Volume 88(1) - 2015

Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale

86th SIBS National Congress

Palermo, Italy, 24-25 October 2013

Botanical Garden, Lanza Hall

jbr

www.jbiolres.org

pagepress

pagepress

Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale

eISSN 2284-0230

Editorial Staff
Lucia Zoppi, Managing Editor
Claudia Castellano, Production Editor
Tiziano Taccini, Technical Support

Publisher
PAGEPress Publications
via Giuseppe Belli 7
27100 Pavia, Italy
Tel. +39.0382.1751762 – Fax. +39.0382.1750481
info@pagepress.org – www.pagepress.org

SOCIETÀ ITALIANA DI BIOLOGIA SPERIMENTALE
SIBS

86th SIBS National Congress

Palermo, Italy, 24-25 October 2013

Botanical Garden, Lanza Hall

PRESIDENT

Marco Giammanco *(University of Palermo, Italy)*

ORGANIZING COMMITTEE

Stefania Aiello *(University of Palermo, Italy)*

Antonella Cascio *(University of Palermo, Italy)*

Marilena Crescimanno *(University of Palermo, Italy)*

Danila Di Majo *(University of Palermo, Italy)*

Carla Flandina *(University of Palermo, Italy)*

Marco Giammanco *(University of Palermo, Italy)*

Maurizio La Guardia *(University of Palermo, Italy)*

Gaetano Leto *(University of Palermo, Italy)*

Antonio Palma *(University of Palermo, Italy)*

Diego Planeta *(University of Palermo, Italy)*

Giovanni Tomasello *(University of Palermo, Italy)*

Marcello Traina *(University of Palermo, Italy)*

Francesca Maria Tumminello *(University of Palermo, Italy)*

In memory of

Prof. SANTO GIAMMANCO

Full Professor of General Physiology, University of Palermo, Italy

Recent advances in human T lymphocyte biology in space

C. Secchi, C. Crescio, A. Pantaleo, P. Pippia

Department of Biomedical Sciences, University of Sassari, Italy

Immunosuppression during spaceflight is a major barrier to safe long-term human space habitation and travel. Remarkable findings in space have shown that gravity changes affect important cellular mechanisms like proliferation, differentiation, genetic expression, cytoskeletal architecture and motility in lymphocytes, monocytes and other mammalian cells. In particular, several experiments performed in space demonstrated that human T lymphocytes have remarkably reduced mitogenic activation (80-90%), thus implicating gravity as a necessary factor in normal immune function.[1,2] Subsequent space studies using sounding rockets, shuttles and International Space Station (ISS) demonstrated that T cell activation requires tight contacts between each other as well between T cell and monocytes as antigen-presenting cells. We were able to see that cells display autonomous movements and interactions in space.[3] Moreover, we investigated the structure of the cytoskeleton and in particular of tubulin and intermediate filaments of vimentin in Jurkat cells by immunofluorescence technique on the sounding rocket MAXUS 1B. We observed, already 30 min after exposure to microgravity, a significant higher formation of large bundles of filaments, showing that the cytoskeleton undergoes important and immediate changes in microgravity.[4] Again important differences between the actin pattern between 1xg and 0xg in J-111 cells were observed in an experiment on board ISS.[5] Such experiments were accompanied by extensive investigations performed in the ground laboratory by the three-dimensional clinostat, called Random Positioning Machine (RPM). This machine has proven, in the last 15 years, to be a useful tool to simulate low g in the ground laboratory and to prepare space investigations. Next experiments conducted in space and in RPM indicate that there are direct gravitational effects on the genetic expression of IL-2 and its receptor in human T lumphocytes. In our investigation on the IL-2R, we focused our attention on the alfa and beta-chains only, because the gamma-chain is not constitutively expressed. Surprisingly, the expression of the alfa-chain was significantly inhibited whereas the expression of the beta-chain was not influenced by microgravity.[6]

Moreover, experiments in RPM using gene arrays and quantitative RT-PCR demonstrated that induction of 91 genes was altered in simulated microgravity conditions. Promoter region analysis found that the majority of genes downregulated in microgravity were controlled by transcription factors NFkB, CREB, ELK, AP-1 and STAT. The fact that phosphorylation of the linker of activation in T cells (LAT) is not down-regulated in simulated microgravity indicating that cholesterol-rich lipid rafts are not involved in the down-regulation of the transcription factors.[7]

Our LEUKIN spaceflight experiment on board the ISS allowed the evaluation of the global gene expression pattern of human T cells after 1.5 hours of stimulation by ConA and anti- CD28 in order to identify the immediate early genes whose transcription may be inhibited in microgravity conditions. Importantly, an onboard centrifuge was used to generate a 1xg simultaneous control to isolate the effects of microgravity from other variables of spaceflight. Microarray expression analysis after 1.5 hours of activation demonstrated that 0xg and 1xg- activated T cells had distinct patterns of global gene expression and identified 47 genes that were significantly differentially down-regulated by at least 2 fold in microgravity. Expression of many genes involved in mitogenesis, cytokine production, apoptosis, and signal transduction and several key immediate early genes were inhibited in microgravity. In particular, transactivation of Rel/NFkB, CREB, and SRF gene targets were down-regulated. Expression of cREL gene targets were significantly inhibited and transcription of cREL itself was significantly reduced in microgravity. Analysis of gene connectivity indicated that the tumor necrosis factor (TNF) pathway is likely a major early downstream effector pathway inhibited in microgravity and may lead to ineffective pro-inflammatory host defenses against infectious pathogens during spaceflight.[8]

Recently, we studied the influence of altered gravity on expression and function of cytoskeletal proteins, chemokines, cytokines and their rceptors by the experiment STIM (Signal Transduction In Microgravity) on board the sounding rocket Maser 12. The launch took place the 13th of feruary 2012 at Esrange Space Center and the microgravity lasted 390 sec. During the flight, one automed plunger activation mechanism initiated the confluente between the activators (Concanavalin A, anti-CD28, anti-CD3) and the cells (human T lymphocytes), while a second plunger initiated that between fixative (formalin) and activated cells in a subsequent phase. The hypergravity phase during the launch resulted in a down regulation of the IL-2 and CD3 receptor and reduction of tyrosine phosphorylation, p44/42-MAPK phosphorylation and histone H3 acetylation, whereas LAT phosphorylation was increased. Compared to the baseline situation at the point of entry into the microgravity phase, CD3 and IL-2 receptor expression at the surface of non-activated T cells were reduced after 6 min. of microgravity. Importantly, p44/42-MAPK- phosphorylation was also reduced in low gravity. In activated T cells, the reduced CD3 and IL-2 receptor expression recovered significantly during in-flight 1xg conditions, but not during microgravity conditions. Beta-tubulin increased significantly after onset of microgravity until the end of the microgravity phase, but not in the in-flight 1xg condition. The results of STIM experiment suggest that key proteins of T cell signal modules are not severely altered in microgravity conditions. Instead, it can be sup-

Correspondence: Proto Pippia, Department of Biomedical Sciences, University of Sassari, via Muroni 25, 07100 Sassari, Italy.
Tel: +39.329.1710218. E-mail: pippia@uniss.it

Journal of Biological Research 2015; 88:5161

posed that the strong T cell inhibiting signal occurs downstream from membrane proximal signaling, such as at the transcriptional level. However, this study could identify signal molecules, which are sensitive to altered gravity, and indicates that gravity is obviously not only a requirement for transcriptional processes as described before, but also for specific phosphorylation/dephosphorylation of signal molecules and surface receptor dynamics.[9]

Future researches in space and in simulated microgravity conditions should focus on delineating the specific mechanisms of how microgravity causes dysregulation of these signal transduction pathways in order to further clarify the molecular basis of spaceflight immunosuppression. Moreover, these findings suggest that the alterations of single cell behaviour observed in the absence of gravity may be exploited for biotechnological and biomedical applications.

References

1. Cogoli A, Tschopp A, Fuchs-Bislin P. Cell sensitivity to gravity. Science 1984;225:228-30.
2. Cogoli A, Bechler B, Cogoli-Greuter M, et al. Mitogenic signal transduction in T lymphocytes in microgravity. J Leukoc Biol 1993; 55:569-75.
3. Cogoli-Greuter M, Meloni MA, Spano A, et al. Movements and interactions of leukocytes in microgravity. J Biotechnol 1996;47: 279-87.
4. Cogoli-Greuter M, Spano A, Sciola L, et al. Influence of microgravity on mitogen binding, motility and cytoskeleton patterns of T lymphocytes and Jurkat cells. Experiments on sounding rockets. J Aerospace Env Med 1998;35:27-39.
5. Meloni MA, Galleri G, Pani G, et al. Space flight affects motilità and cytoskeletal structures in human monocyte cell line J-111. Cytoskeleton 2011;68:125-37.
6. Walther I, Cogoli A, Pippia P, et al. Human immune cells as space travelers. Eur J Med Res 1999;4:361-3.
7. Boonyaratanakomkit JB, Cogoli A, Li CF, et al. Key gravity-sensitive signaling pathways drive T-cell activation. FASEB J 2005;19: 2020-42.
8. Chang TT, Walther I, Li CF, et al. The Rel/NFkB pathway and transcription of immediate early genes in T cell activation are inhibited by microgravity. J Leuk Biol 2012;92:1133-45.
9. Haushild S, Tauber S, Crescio C, et al. Signal transduction in primary human T lymphocytes – results of the MASER-12 suborbital space flight mission. Cell Communication and Signaling 2013;11:32.

The adipokine leptin: a pleiotropic molecule in the human respiratory tract

A. Bruno,[1] M. Giammanco,[2] M. Gjomarkaj,[1] E. Pace[1]

[1]Institute of Biomedicine and Molecular Immunology, National Research Council, Palermo; [2]Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, Italy

Leptin, a 16-kd adipocyte-derived hormone originally described in metabolism regulation, plays a pleiotropic role in the immune system and inflammation.[1] Leptin exerts its action through the leptin receptor (Ob-R), present in several tissues, human respiratory tract included (Figure 1). Leptin is a survival cytokine for human neutrophils and eosinophils,[2,3] other than for other cytotypes, included lung carcinoma cells.[4] The following findings highlight the specific role of leptin both in the lung and in the nasal tract. We firstly find that *ex-vivo* leptin expression is increased and co-localized with lymphocytes T inflammatory cells, in bronchial mucosa of chronic obstructive pulmonary disease (COPD) patients and it is associated with COPD severity, airway inflammation and airflow obstruction.[5] On the other side, previous our *in vitro* and *ex-vivo* results show that the leptin/leptin receptor pathway is decreased in the bronchial epithelium of subjects with mild, uncontrolled, untreated asthma, whereas RBM thickness and TGF-beta 1 are increased, when compared with healthy volunteers.[6] In addition, in another our *in vitro* study, we assess that leptin increases adenocarcinoma cell line proliferation and the pathway with its receptor is increased by the flavonoid apigenin (4,5,7,-trihydroxyflavone).[7] Furthermore, our recent *in vitro* results report that the leptin/leptin receptor pathway is involved in human nasal epithelial homeostasis in allergic rhinitis and its expression is restored by Fluticasone Furoate in presence of the allergens.[8] In conclusion, in the submucosa, leptin might act as a cytokine-like mediator capable of playing a role in airway inflammation in chronic obstructive pulmonary disease with a potential impact on the severity of the disease; in the epithelium, the leptin/leptin receptor pathway is involved both in airway and in nasal epithelial homeostasis, in asthma and in allergic rhinitis, promoting also, in a cancer context, epithelial cell proliferation. Its expression decreases in subjects with uncontrolled and severe asthma and in presence of allergen exposure and is inversely correlated with airway remodelling, and cancer cell apoptosis.

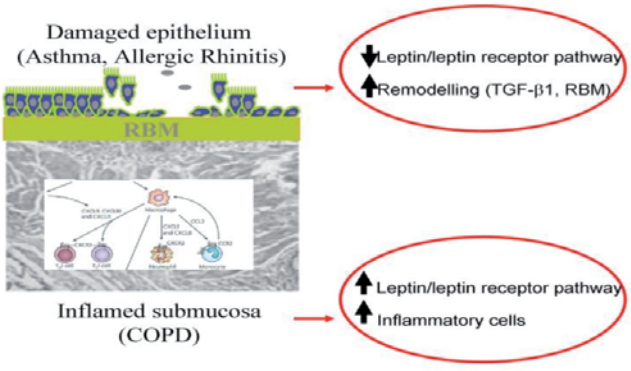

Figure 1. Leptin in human respiratory tract.

Correspondence: Andreina Bruno, Institute of Biomedicine and Molecular Immunology, National Research Council, via Ugo La Malfa 153, 90146 Palermo, Italy.
E-mail: andreinabrn@gmail.com

Journal of Biological Research 2015; 88:5161

References

1. Matarese G, Procaccini C, De Rosa V, et al. Regulatory T cells in obesity: the leptin connection. Trends Mol Med 2010;16:247-256.
2. Bruno A, Conus S, Schimd I, Simon HU. Apoptotic pathways are inhibited by leptin receptor activation in neutrophils. J Immunol 2005;174:8090-6.
3. Conus S, Bruno A, Simon HU. Leptin is an eosinophil survival factor. J Allergy Clin Immunol 2005;116:1228-34.
4. Terzidis A, Sergentanis TN, Antonopoulos G, et al. Elevated serum leptin levels: a risk factor for non-small-cell lung cancer? Oncology 2009;76:19-25.
5. Bruno A, Chanez P, Chiappara G, et al. Does leptin play a cytokine-like role within the airways of COPD patients? Eur Respir J 2005;26:398-405.
6. Bruno A, Pace E, Chanez P, et al. Leptin and leptin receptor expression in asthma. J Allergy Clin Immunol 2009;124:230-7.
7. Bruno A, Siena L, Gerbino S, et al. Apigenin upregulates leptin/leptin receptor pathway and induces cell apoptosis in lung adenocarcinoma cell line. Eur J Cancer 2011;47:2042-51.
8. Bruno A, Gerbino S, Ferraro M, et al. Fluticasone furoate maintains epithelial homeostasis via leptin/leptin receptor pathway in nasal cells. Eur Respir J 2013;42(Suppl.57):1009s.

K:D-Rib on biology of human cancer and not cancer cell line

L. Bruni,[1-3] S. Croci[1,2]

[1]Unit of Biophysics and Medical Physics, Department of Neuroscience, University of Parma; [2]National Institute of Biostructures and Biosystems, Rome, Italy; [3]Valsè Pantellini Foundation, Oviedo, Spain

This work describes different effects of K:D-Rib solution treatment: from one side the slow down of cell proliferation and the reduction of chemoinvasive potential of human breast cancer cell line (HTB-126) and from the other the maintenance of normal proliferation and normal morphology in mammary human not cancer epithelial cell line (HTB-125). K:D-Rib is a water solution of D-ribose and $KHCO_3$. The role of D-ribose on the energetic metabolism and its involvement into glycogen synthesis,[1,2] as well as the importance of K^+ into the cell physiology, are well known.[3,4] It has been found that K^+ is essential to fold and to stabilize G-quadruplex[5] with a strong relevance for telomeric structures[2,6] and for oncogenic promoter regions.

Our results showed that K:D-Rib has a cytostatic effect on canine carcinoma cell line (A72), slows the colony formation ability of the HTB-126 cell line and has an antioxidant behaviour reducing MTT salt to formazan in absence of cells.[1] These results are confirmed by our most recent work, demonstrating that 5mM K:D-Rib increase the cell cycle time of HTB-126 cell line treated with K:D-Rib at the concentration of 5mM, from 44h to 59h. Here it will be show how K:D-Rib interferes both on HTB-126 cell line proliferation and cell morphology. Results on cell morphology using Atomic Force Microscopy (AFM) are presented. K:D-Rib is tested also on human mammary epithelial cell line (HTB-125). HTB-125 cells treated with 5 mM K:D-Rib do not display toxicity or notable cell proliferation decreasing rate compared to the control one. HTB-125 cell morphology is analyzed by AFM. As mentioned before a key point of cancer cells is the capability to invade tissues nearby or far from cancer formation site. Tumour motility is an important step in the intricate process leading to the formation of metastasis. It has been shown that metastatic cells are more motile than non-metastatic tumour cells and most motile of normal cells. Metastatic cells lose growth-inhibitory responses, undergo alterations in adhesiveness and demonstrate enhanced production of enzymes that can degrade extracellular matrix components. Since the development of metastatic disease in breast cancer is one of main responsible of cancer mortality, the stopping and the understanding of the mechanisms that facilitate metastatic tumour progression is of prime importance.[7] We have investigated if K:D-Rib solution within 9 days can modify the migration and the invasion ability. The experiments show HTB-126 cells are able to migrate across the coverslip toward the FBS – agar spot and to invade it within 48, but the relative cell number inside the AGAR-FBS decrease already after five days of treatment. After nine days of treatment with K:D-Rib the relative cell number, inside the AGAR-FBS spot is reduced to 25%, demonstrating that tumorigenic potential is highly decreased with K:D-Rib treatment. These results show that 5mM K:D-Rib causes the change of some aspects like migration, invasion and proliferation of HTB-126 cell line. Despite these evidences K:D-Rib does not interfere neither with the proliferation of HTB-125 cell line nor with cell morphology.

Correspondence: Luca Bruni, Unit of Biophysics and Medical Physics, Department of Neuroscience, University of Parma, via Volturno 39/E, 43125 Parma, Italy.
E-mail: lucabr77@gmail.com

Journal of Biological Research 2015; 88:5161

References

1. Croci S, Bruni L, Bussolati S, et al. Potassium bicarbonate and D-ribose effects on A72 canine and HTB-126 human cancer cell line proliferation in vitro. Cancer Cell Int 2011;11:30.
2. Heiden MGV, Cantley LC, Thompson CB. Understanding the warburg effect: the metabolic requirements of cell proliferation. Science 2009;324:1029-33.
3. Dai JX CM, Yang DZ. Polymorphism of human telomeric quadruplex structures. Biochimie 2008;90:1172-83.
4. Zhou FS, Tian Y, Youngbull C, et al. A new highly selective fluorescent K+ sensor. Journal of the American Chemical Society 2011:18530-3.
5. Parkinson GN, Lee MPH, Neidle S. Crystal structure of parallel quadruplexes from human telomeric DNA. Nature 2002;417:876-80.
6. Lipps HJ, Rhodes D. G-quadruplex structures: in vivo evidence and function. Trends in Cell Biology 2009;19:414-22.
7. Fernandis AZ, Prasad A, Band H, et al. Regulation of CXCR4-mediated chemotaxis and chemoinvasion of breast cancer cells. Oncogene 2004;23:157-67.

Does the exercise training intensity affect plasmatic redox status in rhythmic gymnastics?

M. Bellafiore,[1,2] G. Battaglia,[1,2] G. Caramazza,[1,2] M. Petrucci,[1,2] M. Giaccone,[1,2] A. Bianco,[1,2] A. Palma[1,2]

[1]Department of Legal, Social and Sports Sciences, University of Palermo; [2]Regional Sport School of CONI Sicily, Palermo, Italy

Introduction

It is known that factors such as exercise intensity,[1] length[2] and type[3] can modify the plasmatic oxidant/anti-oxidant production. In rhythmic gymnastics, adolescent female athletes showed a higher lipid peroxidation[4] and an altered antioxidant enzyme profile compared with their untrained peers.[5] The aim of this study was to assess whether the plasmatic H_2O_2 levels and antioxidant capacity were affected by the exercise training intensity in rhythmic gymnastics coaches.

Materials and Methods

Ten women (age: 23.8±3.42 y; weight: 52.58±4.57 kg; height: 158.42±2.20 cm; body mass index: 20.88±1.23), with 13.14±5.40 years of practice in rhythmic gymnastics and coaches from at least 4 years at a competitive level, voluntarily participated into this study. One week before training, trainers performed a laboratory graded exercise test on the treadmill in order to determine their maximal heart rate (HR_{max}), maximal oxygen consumption (VO_{2max}) and anaerobic threshold (AT). Two interval-training sessions, separated by 48 hours of recovery, were performed with different intensities. The first was carried out at a low-moderate intensity; while the second at a high intensity. Both lasted 45 minutes and consisted of exercises aimed to develop anaerobic power, strength, flexibility and body balance. Before cool down, two performances of competition technical skills coordinated to the music were also executed. During training, HR was continuously monitored with 'Polar team' system. Immediately before and after the training session, blood samples were taken from fingertip's coaches and H_2O_2 levels and antioxidant capacity were measured through reactive oxygen metabolites (dROMs) and biological antioxidant potential (BAP) test, respectively. Newman-Keuls multiple comparison test was used for evaluating the significant differences. Alpha level for significance was set to $P<0.05$.

Correspondence: Marianna Bellafiore, Department of Legal, Social and Sports Sciences, University of Palermo, via Maqueda 172, 90133 Palermo, Italy.
Tel: +39.091.23892001 - Fax: +39.091.329355.
E-mail: marianna.bellafiore@unipa.it

Journal of Biological Research 2015; 88:5161

Results

Coaches executed the first training session at an average intensity of 66% HR_{max}, mainly in aerobic condition and only 5.5% of total time in anaerobic condition; while in the second session they spent 42% of total time at an intensity ranging from 80 to 100% HR_{max} and for 25% above the anaerobic threshold. After low-moderate intensity training, H_2O_2 levels were significantly lower than baseline and they came back to baseline following 48 h of recovery. After high intensity training, H_2O_2 amount slightly decreased compared with baseline ($P>0.05$); while it was significantly higher than after low-moderate intensity training. All these values corresponded to a middle oxidative stress when compared with a standard range.[6] Antioxidant capacity did not change following low-moderate intensity training, while it significantly increased after 48 h of recovery. In contrast, it significantly decreased in response to high intensity training reaching the values obtained after low-moderate intensity training.

Conclusions

These results show that training intensity has different effects on ROS production and antioxidant capacity in rhythmic gymnastics. In detail, a low-moderate intensity session induces H_2O_2 production; while a high intensity session negatively affects the antioxidant defences.

Therefore, it would be appropriate to introduce a anti-oxidant diet or supplementation for protecting rhythmic gymnastics trainers by oxidative stress.

References

1. Bloomer RJ, Goldfarb AH, Wideman L, et al. Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. J Strength Cond Res 2005;19:276-85.
2. Bogdanis GC, Stavrinou P, Fatouros IG, et al. Short-term high-intensity interval exercise training attenuates oxidative stress responses and improves antioxidant status in healthy humans. Food Chem Toxicol 2013;61:171-7.

3. Cubrilo D, Djordjevic D, Zivkovic V, et al. Oxidative stress and nitrite dynamics under maximal load in elite athletes: relation to sport type. Mol Cell Biochem 2011;355:273-9.
4. Guerra A, Rego C, Laires MJ, et al. Lipid profile and redox status in high performance rhythmic female teenagers gymnasts. J Sports Med Phys Fitness 2001;41:505-12.
5. Alshammari E, Shafi S, Nurmi-Lawton J, et al. Altered antioxidant and trace-element status in adolescent female gymnasts. Int J Sport Nutr Exerc Metab 2010;20:291-8.
6. Alberti A, Bolognini L, Macciantelli D, Carratelli M. The radical cation of N,N-diethyl- para-phenylendiamine: a possible indicator of oxidative stress in biological samples. Res Chem Intermed 2000;26:253-67.

Heart rate recovery after exercise and maximal oxygen uptake in sedentary patients with type 2 diabetes

A. Cataldo, D. Cerasola, D. Zangla, P. Proia, G. Russo, R. Lo Presti, M. Traina

Department of Sports Sciences, University of Palermo, Italy

Aims

Heart rate recovery after exercise (HRR) is an estimate of autonomic modulation of the heart, and has been shown to be inversely associated with insulin resistance, metabolic syndrome, and type 2 diabetes.[1] Type 2 diabetes is associated with poor exercise tolerance and maximal aerobic capacity (VO_2max).[2] Aim of our study was to assess the relationship between HRR and VO_2max in sedentary patients with type 2 diabetes.

Methods

Maximal treadmill exercise testing using standard or modified Bruce protocol was performed in 16 (8 males and 8 females) sedentary patients with type 2 diabetes (T2D), and in 16 (9 males and 7 females) age-matched sedentary non-diabetic controls (ND). HRR (bpm) was defined as the difference between maximum heart rate during the exercise test and heart rate 2 minutes after cessation of the exercise (Figure 1). The recovery protocol consisted of walking on treadmill at 2.0 km/h of speed and 0% of grade. Oxygen uptake was recorded and VO_2max (mL/kg/min) was defined as the highest 30 seconds average achieved during the test. For the statistical analysis of the data, Student's t-test for independent samples and linear regression analysis were used.

Results

The characteristics of subjects are shown in Table 1. The two groups were similar in age and body weight. BMI was higher in T2D (30.1±3.6 *vs* 26.9±4.2, P=0.029). VO_2max was significantly lower in T2D compared to ND (20.6±8.4 *vs* 28.2±8.1 mL/kg/min, P=0.002) and, according to *Normative Table by age and gender* from ACMS, the aerobic capacity was classified very poor in all T2D and in 11/16 of ND. HRR was significantly lower in T2D (28±8.4 *vs* 37±8.9 bpm, P=0.008). A significant correlation between HRR and VO_2max has been found in both T2D (Figure 2) and ND (r=0.672, P=0.004 and r=0.620, P=0.010 respectively).

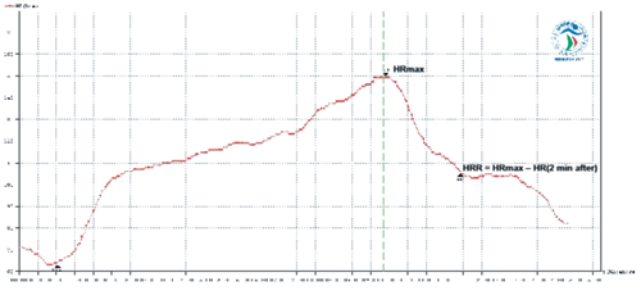

Figure 1. Example of heart rate recovery phase analysis after a maximum reached heart rate.

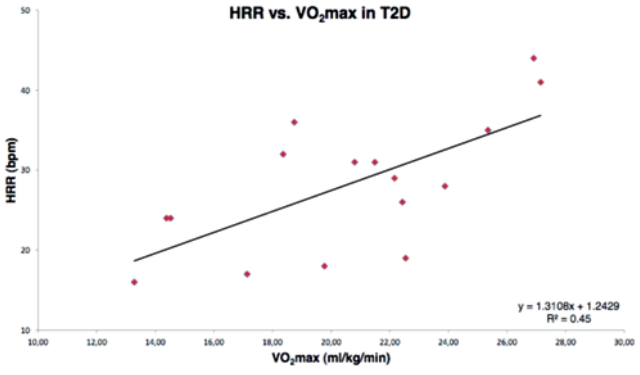

Figure 2. Correlation between heart rate recovery and VO_2max in type 2 diabetes patients.

Table 1. Characteristics of subjects.

	T2D (n=16) Mean±SD	Control (n=16) Mean±SD	P
Age (years)	57±7.5	53±6.9	0.100
Height (cm)	165±7.7	166±9.8	0.826
Weight (kg)	82±10.7	74±15.5	0.119
BMI	30.1±3.6	26.9±4.2	0.029
HRmax (bpm)	130±16.1	143±13.5	0.024
VO_2max (mL/kg/min)	20.6±4.3	28.2±8.1	0.002
HRR (bpm)	28±8.4	37±8.9	0.008

T2D, type 2 diabetes; HRR, heart rate recovery.

Correspondence: Angelo Cataldo, Department of Sports Sciences, via E. Duse 2, Palermo, Italy.
Tel: +39.091.23865500.
E-mail: angelo.cataldo@unipa.it

Journal of Biological Research 2015; 88:5161

Conclusions

The results of our study showed that both HRR and VO_2max were significantly reduced in T2D *versus* ND. The positive linear correlation between HRR and VO_2max suggests that in T2D the heart rate recovery after exercise, index of autonomic modulation, might improve in response to a training aimed to increase aerobic capacity.

References

1. Sacre JW, Jellis CL, Coombes JS, Marwick TH. Diagnostic accuracy of heart-rate recovery after exercise in the assessment of diabetic cardiac autonomic neuropathy. Diabet Med 2012;29:e312-20.
2. Colberg SR, Sigal RJ, Fernhall B, et al. Exercise and type 2 diabetes: ACSM and ADA joint position statement. Diabet Care 2010;33:e147-67.

Meccanismi immunologici e molecolari del danno epatico da farmaci

A. Licata,[1] S. Aiello,[2] V. Calvaruso,[1] P.L. Almasio[1]

[1]Gastroenterology Unit, Department of Internal and Specialized Medicine, University of Palermo; [2]Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, Italy

Il fegato è la centrale metabolica del nostro organismo. I farmaci, in seguito alle reazioni di fase I e II vengono trasformati in metaboliti attivi meno tossici, ma idrofili pronti per essere eliminati. Il sistema responsabile di questa trasformazione è il citocromo P450 presente nel reticolo endoplasmatico liscio dell'epatocita. In seguito, intervengono le glucoronosil-tranferasi, le sulfotransferasi e le GSH-transferasi che idrolizzano definitivamente il composto. Il trasporto del metabolita del farmaco fuori dall'epatocita avviene attraverso il polo biliare ed è mediato da un sistema di trasportatori di membrana, l'ATP Binding Cassette (ABC) Transporter Superfamily. L'alterazione dell'attività dei trasportatori, a diversi livelli, molecolare e trascrizionale, è uno dei meccanismi responsabile di epatotossicità.

I polimorfismi genetici e/o i fattori ambientali, ad esempio l'alcol e/o i farmaci concomitanti, contribuiscono alla suscettibilità individuale nel determinare il danno epatico da farmaci. L'HLA si è dimostrato essere uno dei più importanti predittore di suscettibilità individuale anche per quei farmaci per i quali questa non era stata mai sospettata. A questo proposito, è stato dimostrato che c'è una forte associazione tra il danno epatico indotto da flucloxacillina e l'allele HLA B* 5701, e tra aplotipi HLA di classe II e danno epatico da amoxicillina-clavulanico e ximelagatran.

I farmaci, in generale, possono avere un effetto diretto sugli epatociti o suscitare una reazione immune che può essere di due tipi: innata o adattativa. Nella maggior parte dei casi, la bioattivazione di un farmaco porta ad un metabolita reattivo che determina una disfunzione mitocondriale con conseguente riduzione dei livelli di ATP, disaggregazione del citoscheletro e quindi rottura della membrana cellulare epatocitaria. I metaboliti attivi influenzano il trasporto delle proteine (MDR-3) attraverso il polo biliare della membrana eritrocitaria determinando l'interruzione del flusso biliare, il blocco di escrezione della bilirubina e infine la colestasi.

In alternativa ad una azione diretta sulla membrana cellulare, lo stress epatocitario determina l'attivazione del sistema immune innato attraverso le cellule natural killer (NK) del fegato che secernono interferone-gamma (IFNγ) ed interleuchina (IL)-4, e sono in grado di uccidere direttamente le cellule tramite il sistema Fas/FasLigand. Cellule di Kupffer ed NK contribuiscono alla progressione del danno epatico producendo mediatori pro-infiammatori (citochine, chemochine, ROS); questi possono avere azione citotossica diretta (perossido d'idrogeno, ossido nitrico) degradando la matrice extracellulare, oppure promuovendo l'adesione e l'infiltrazione cellulare dei leucociti polimorfonucleati.

Nella patogenesi del danno epatico è coinvolto anche il sistema immune adattativo. Il metabolita reattivo può infatti legarsi in modo covalente ed alterare le proteine del fegato, promuovendo l'attivazione delle cellule T citotossiche e la produzione di citochine (reazione immuno- mediato). Il meccanismo del danno da farmaci immuno mediato non è ben chiaro, e comporta un'azione *apten-like*. Generalmente infatti le sostanze chimiche a basso PM non sono immunogeni ma possono diventare tali quando sono legati ad una macromolecola, come una proteina. Se un metabolita attivo di un farmaco prodotto dal citocromo P450 è in grado di agire come un aptene, e si lega covalentemente ad una proteine del fegato, il sistema immunitario la percepirà come *non-self* causando una reazione autoimmune. Il risultato di questi eventi, sia attraverso una reazione diretta sulla membrana cellulare, sia attraverso l'induzione di una risposta immunitaria, è la morte cellulare: necrosi o apoptosi.

L'induzione dell'apoptosi piuttosto che la necrosi dipende da diversi fattori, tra cui lo stato energetico (ATP). Una lesione grave per i mitocondri determina deplezione energetica della cellula, che perde la regolazione osmotica e va in necrosi. Una lesione meno grave senza importante deplezione di ATP è in grado di mantenere la regolazione osmotica e porta all'apoptosi. La necrosi epatocellulare è l'evento principale di cui è responsabile il danno epatico da farmaci; ne possono essere bersaglio sia cellule endoteliali che quelle dei dotti biliari. In fatti distinguiamo il danno epatico da farmaci di tipo epatocellulare (nimesulide), di tipo colestatico (amoxicillina clavulanico) e misto.

Correspondence: Anna Licata, Gastroenterology Unit, Department of Internal and Specialized Medicine, University of Palermo, Piazza delle Cliniche 2, 90127 Palermo, Italy.
Tel: +39.091.23867516.
E-mail: anna.licata@unipa.it

Journal of Biological Research 2015; 88:5161

Identification and sustainable exploitation of wild edible mushrooms in rural areas (*Mycoticon*, LdV-ToI project): development of an innovative training package to meet educational and income-generating demands in South Europe and to improve the use of mushrooms as high-value food

G.I. Zervakis,[1] G. Venturella,[2] C.M. Denchev,[3] P.T. Fitsilis,[4] V.C. Gerogiannis,[4] S. Papaefthimiou,[5] J. Georgi,[6] A. Saitta,[2] E. Polemis,[1] T.T. Denchev,[5] B. Assyov,[5] M.L. Gargano[2]

[1]Laboratory of General and Agricultural Microbiology, Agricultural University of Athens, Greece; [2]Department of Agricultural and Forest Sciences, University of Palermo, Italy; [3]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria; [4]Department of Project Management, Technological Education Institute of Thessalia, Larissa; [5]KEK Aegeas, Larissa, Greece; [6]Neapolis University of Pafos, Cyprus

In Mediterranean and in southeast Europe the activities of a significant part of the population are traditionally linked with agriculture, forestry and animal husbandry. However, many rural communities are experiencing serious difficulties associated with low income per person and poor employment prospects combined with increased demographic decline. Alternative activities such as the collection and trading of wild edible mushrooms as well as the cultivation of choice species could contribute at providing valuable solutions both in financial and environmental terms.

The total number of fungal species which are considered having edible and/or medicinal value is over 2300.[1] Most of them form large conspicuous sporophores (*i.e.* mushrooms) during their life-cycle, which are either harvested from the wild or cultivated on a wide range of plant and agro-industrial residues and by-products. Foraying and picking of wild edible mushrooms has a long tradition in most European countries; therefore it constitutes a significant socioeconomic activity, while at the same time reflects local knowledge and social practices that are worth preserving. Recent food market tendencies reveal a high demand potential for wild edible mushrooms among urban consumers. In those cases that wild fungi are not well-known because pertinent knowledge was not spread within families or local communities, people avoid their harvest; instead they are oriented at consuming cultivated mushrooms which become increasingly popular. This latter type of activity is tightly associated with environmental protection through recycling and valorization of low-value substrates together with the conservation of some highly sought-after mushroom species.[2,3]

The *Mycoticon* project (EU, LdV-ToI) involves Universities, Technological, and Research Institutions as well as local stakeholders and associated end-users from four European countries, *i.e.* Bulgaria, Cyprus, Greece and Italy. These partners combine their experience and expertise at developing an integrated educational and training package together with its respective tools to meet the demands of suitable target-groups willing to create collective entrepreneurship schemes for exploiting the economic potential of wild mushrooms in rural areas. Ultimately, the objective is to facilitate the generation of a new source of non-subsidized income and create new jobs in areas desperately in need of both. In parallel, local people are expected to be presented with incentives to adopt sustainable management and harvesting practices for wild edible mushrooms together with basic knowledge on mushroom cultivation.

Among other anticipated deliverables, national reports were compiled for each participating country as regards the current knowledge/situation on diversity, harvest and trade of wild edibl mushrooms as well as on commercial mushroom production. In addition, a voluminous textbook was prepared[4] which provided a detailed description of 22 choice edible and 11 selected poisonous mushrooms (together with many other related taxa) of significance in all four countries. Moreover, it included general information about biology and ecology of mushroom fungi, their common habitats/ecosystems, proper harvest practices and suitable food preservation methods, relevant legislation and conservation issues, and basic guidelines for the cultivation of the most popular species together with prospects for developing tourism activities associated with mushrooms. All of them formed the basis for the development of an innovative training material established both on paper and online by creating a moodle web-page (http://moodle.teilar.gr/). This electronic tool was assembled in four languages (English, Italian, Greek and Bulgarian) and it now provides a user-friendly and flexible modular training course through which e-self-assessment and e-accreditation could be also accomplished. The training package complies with EQF rules and it will be further structured according to EC-VET provisions. Its content is anticipated to enhance the development of pertinent skills and subsequently increase employment of qualified people in rural areas. Furthermore, it provides the prerequisites for combining local assets and resources into mushroom products that meet consumers' expecta-

Correspondence: Georgios I. Zervakis, Laboratory of General and Agricultural Microbiology, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece.
Tel: +30.210.5294341.
E-mail: zervakis@aua.gr

Acknowledgements: this work was performed in the frame of the *Identification and sustainable exploitation of wild edible mushrooms in rural areas* (*Mycoticon*) project, and it was funded by the European Union (Leonardo da Vinci – Transfer of Innovation).

tions. Such activities constitute a highly recommended approach in Europe since rural income could derive from integrated direct and indirect recourses (by also supporting conservation and environmental sustainability) and not only by the primary agricultural production.

References

1. Marshall E, Nair NG. Make money by growing mushrooms, p. 64. Rome, Italy: Food and Agriculture Organization of the United Nations; 2009.
2. Gargano ML, Zervakis GI, Venturella G. *Pleurotus nebrodensis*, a very special mushroom, p. 150 (e-book). The Netherlands: Bentham Science Publishers; 2013.
3. Zervakis G, Venturella G. Mushroom breeding and cultivation favors ex situ conservation of Mediterranean *Pleurotus* taxa. In: Engels JMM, Ramanantha Rao V, Brown AHD, Jackson MT, eds. Managing plant genetic diversity. UK: IPGRI-CABI Publishing; 2002. pp. 351-358.
4. Denchev C, Venturella G, Zervakis G. Identification and sustainable exploitation of wild edible mushrooms in rural areas. Thessalia, Greece: Technological Education Institute of Thessalia; 2013.

The acute phase response of Atlantic cod (*Gadus Morhua* L.): expression of immune response genes after infection with *Aeromonas Salmonicida* subsp. *Achromogenes*

A. Fazio,[1] B.T. Bragason,[2] B. Magnadottir,[2] C. Faggio,[1] S. Gudmundsdottir[2]

[1]Department of Biological and Environmental Sciences, University of Messina, Italy; [2]Institute for Experimental Pathology, University of Iceland, Reykjavik, Iceland

The Acute Phase Response (APR) is a core part of the innate immune response and is present in all animal species including mammals, fish and invertebrates. APR is a reaction of the body to injury, trauma or infection and the basic aim is to restore homeostasis. The APR can appear as a local reaction but mainly as systemic reactions including increased secretion of some hormones, activation of the complement system and increased synthesis and secretion of Acute Phase Proteins (APP).[1] Approximately 40 plasma proteins have been defined as APP.[2] Those proteins can have various functions and change in their plasma levels. APP's are classified as positive when their plasma concentration increases and negative when their plasma concentration decreases following the APR. Studies suggest that fish have an APR response that is delayed compared to mammals.[3,4] APR has been studied in several fish species and a large number of APPs have been identified in fish. In this study, APR was induced in Atlantic cod (*Gadus morhua L.*) by intramuscular injection of *Aeromonas salmonicida subsp. achromogenes* (Asa), a common bacterial pathogen in cod and other fish species, causing atypical furunculosis.[5] Asa is endemic in Iceland and caused serious losses of farmed salmonids before vaccination became a common practice.[6] Commercial Asa vaccines are as yet not available for cod and experimental vaccines show variable and often poor protection.[7] The aim of the current study was to examine the acute phase induced by Asa by mesuring the gene expression in cod's kidney and spleen as well as cortisol levels in serum. Cod, mean weight 97.3 g, were divided into three groups: two groups received intramuscular injections of two different concentrations of Asa and the third, a control group, was injected intramuscularly with PBS. Kidney and spleen were sampled from seven fish from each group at time 0 before treatment and at 1 hour, 24 hours, 72 hours and 168 hours after the injection. Blood was collected from the caudal vessel. An ELISA assay (Neogen Corp KY,USA) was used to measure cortisol. For the gene expression analysis of IL-1β, hepcidin and transferrin, total RNA was isolated from tissue samples from kidney and spleen with a NucleoSpin® RNA II kit following the manufacturer's instructions (Macherey-Nagel). Complementary DNA (cDNA) was prepared with the Revertaid™ First Strand cDNA Synthesis Kit, according to the manufacturer's instructions (Thermo). Quantitative real time PCR (qPCR) analyses were performed on a StepOne Plus™ real time PCR instrument (Applied Biosystems). The gene expression data were normalized to the expression of the reference genes ubiquitin or RPL*4* with identical results. The expression of the reference genes in the tissue samples used was relatively stable. The results for gene expression in the controls and Asa injected fish were compared at each time point. Overall, the gene expression results showed a stronger response in the spleen than in the kidney. In Asa injected fish there was a significant increase in IL-1β and hepcidin gene expression at 24h, compared to controls, in both organs. Transferrin gene expression was also significantly elevated in both organs, reached a maximum peak at 72h in the kidney and at 168h in the spleen (Table 1). The results of cortisol analysis showed a statistically significant increase of cortisol levels with a peak at 72h after injection (Figure 1). At the end of the experiment the cortisol levels were significantly elevated compared to the control fish. IL-1β is one of the earliest pro-inflammatory cytokines to respond to infection and induces a cascade of reactions leading to inflammation.[8] The observed early increase in IL-1β gene expression following an acute phase induction with Asa was in agreement with other studies in fish. The serum cortisol levels observed in this study reached a maximum concentration at 72h when the IL-1β gene expression had started to decrease. This could mean that cortisol had a role in the suppression of the IL-1β gene expression as described by other.[9] Transferrin is the major iron binding protein and hepcidin is an

Correspondence: Bergljot Magnadottir, Institute for Experimental Pathology, University of Iceland, Keldur, Keldnavegur 3, 112-Reykjavik, Iceland.
E-mail: bergmagn@hi.is

Acknowledgements: this research was supported by AVS R&D Found of Ministry of Fisheries and Agriculture in Iceland.

Table 1. Expression patterns of the genes IL-1β, hepcidin and transferrin of both organs at main time points. The max (maximum) indicates the time point where each gene reached the maximum peak.

Gene	Organ	24 h	72 h	168 h
IL-1β	Spleen	⇑ (max)	⇓	⇓
	Kidney	⇑ (max)	⇓	⇓
Hepcidin	Spleen	⇑ (max)	⇓	⇓
	Kidney	⇑ (max)	⇓	⇓
Transferrin	Spleen	⇑	⇑	⇑ (max)
	Kidney	⇑ (max)	⇑	⇓

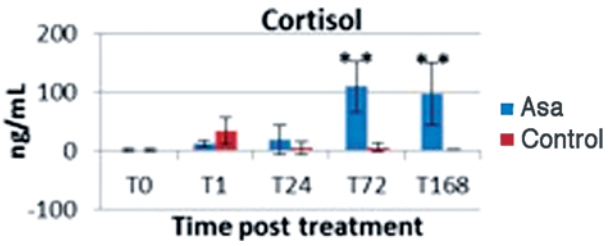

Figure 1. Bar graphs that shows the mean (+/-SD) serum cortisol concentration of untreated control fish or fish injected i.m with Asa sampled at different times after injection. The asterisks indicate a significant difference (P<0.001) in the cortisol levels of the Asa treated fish compared to the control fish at the same time point.

antimicrobial peptide as well as an APP and both have central roles in the iron metabolism of the host. Iron acquisition is important during bacterial infections as it is essential for bacterial growth. The host responds by increasing gene expression of hepcidin and transferrin, especially in the spleen. In conclusion: Infection by Asa resulted in a significant increase in the stress hormone cortisol in the early stages of infection that stayed high until the end of the experiment. Hepcidin and IL-1β showed a strong response in spleen, with similar curves in both organs. Transferrin expressions increase significantly at 24 hours in both organs and remained significantly elevated throughout the experiment. The next steps will include gene expression in liver samples and iron levels measuring in serum at each time point.

References

1. Bayne CJ, Gerwick L. The acute phase response and innate immunity of fish. Dev Comp Immunol 2001;25:725-43.
2. Black S, Kushner I, Salmols D. C-reactive protein. J Biol Chem 2004; 279:48487-90.
3. Russel S, Hayes MA, Simko E, Lumsden JS. Plasma proteomic analysis of the acute phase response of rainbow trout (Oncorhynchus mykiss) to intraperitoneal inflammation and LPS injection. Dev Comp Immunol 2006;30:393-406.
4. Magnadottir B, Audunsdottir SS, Bragason BT, et al. The acute phase response of Atlantic cod (*Gadus morhua L.*): humoral and cellular response. Fish Shellfish Immunol 2011;30:1124-30.
5. Magnadottir B, Bambir SH, Gudmundsdottir BK, et al. A typical Aeromonas salmonicida infection in naturally and experimentally infected cod (*Gadus morhua L.*). J Fish Dis 2002;25:583-97.
6. Guðmundsdottir BK. Infections by atypical strains of the bacterium Aeromonas salmonicida. Iceland Agr Sci 1998;12:5512-23.
7. Guðmundsdottir BK, Bjornsdottir B. Vaccination against atypical furunculosis and winter ulcer disease of fish. Vaccine 2007;25: 5512-23.
8. Seppola M, Larsen AN, Steiro K, et al. Characterisation and expression analysis of the interleukin genes, IL-1β, Il-8 annd IL-10, in Atlantic cod (*Gadus morhua L.*). Mol Immunol 2008;45:887-97.
9. Zou J, Holland J, Pleguezuelos O, et al. Factors influencing the expression of interleukin-1 beta in cultured rainbow trout (*Oncorhynchus mykiss*) leucocytes. Dev Comp Immunol 2000;24: 575-82.

Effects of *Pelagia Noctiluca* crude venom on cell viability and volume regulation

R. Morabito,[1] A. Marino,[2] S. Dossena,[3] M. Paulmichl,[3] G. La Spada[2]

[1]Department of Human and Social Sciences, University of Messina; [2]Department of Biological and Environmental Sciences, University of Messina, Italy; [3]Institute of Pharmacology and Toxicology, Paracelsus Medizinische Privatuniversitat, Salzburg, Austria

Among Cnidaria, *Pelagia noctiluca*, is one of the most dangerous jellyfish in the Mediterranean Sea, where its blooming has been very abundant for many years. Toxicology of crude venom from *P. noctiluca* stinging cells is reported in this presentation. Both *in vivo* and *in vitro* biological assays have been performed to verify and, possibly, measure the toxicity of *P. noctiluca* crude venom, whose composition is still not completely defined.

At first we tested the hemolytic activity of crude venom from single nematocysts discharged by a chemical non enzymatic method. The delivered venom induced a powerful and rapid hemolytic activity. As a second step, crude venom extracted from a population of isolated nematocysts, provoked a dose-dependent hemolysis in erythrocytes from different sources, including eel, rabbit, chicken and human. Moreover, *P. noctiluca* crude venom directly induced mitochondrial trans-membrane potential ($\Delta\Psi m$) collapse and Reactive Oxygen Species (ROS) generation in SH-SY5Y cells derived from human neuroblastoma.

In order to better characterize the biological effects of the crude venom, *in vivo* assays were also performed. Injection of crude venom into the rat paw evoked an inflammatory reaction in a dose-dependent manner. Immunohistochemical analysis showed a marked acute inflammatory response in the tissues, with accumulation of polymorphonuclear neutrophils. Treatment with melatonin as antioxidant significantly reduced the inflammatory response, thereby confirming that oxidative stress plays a major role in inducing the observed pathological changes.

In addition to hemolytic and cytolytic assays, a test on cell volume regulation capability was also chosen to describe the biological activity of *P. noctiluca* crude venom. As already demonstrated, isolated nematocytes of the sea anemone *A. mutabilis* exhibit Regulatory Volume Decrease (RVD) when stimulated with a 35% hypotonic solution. In nematocytes exposed to different concentrations of crude venom (corresponding to the amount contained in 10, 25 and 50 nematocysts/μL) RVD was partially inhibited 25 nematocysts/μL crude venom concentration and fully blocked at 50 nematocysts/μl completely recovered, therefore indicating that K^+ channels inhibition may account for the venom-induced RVD impairment. RVD tests were also performed on HEK293 Phoenix cells, a human embryonic kidney cell line. In control conditions, the cells stimulated by hypotonicity showed an initial swelling followed by RVD, whereas in 0.025 μg/μL crude venom-containing extracellular hypotonic solution, RVD was dramatically impaired. Furthermore, pre-incubation of cells in a crude venom-containing extracellular isotonic solution prevented RVD after hypotonic stress. Surprisingly, the presence of toxin in the extracellular isotonic solution led to cell swelling even in the absence of an osmotic gradient. This phenomenon was not observed in control conditions and the precise mechanism needs to be further elucidated.

We conclude that *P. noctiluca* crude venom extract has hemolytic activity, pro-inflammatory action, induces mitochondrial potential collapse and ROS production. In addition, crude venom inhibits RVD in both cnidarians and mammalian cells after hypotonic stress and leads to cell swelling in isotonic conditions. Our experiments add novel information to understand the mechanism of action of *P. noctiluca* venom.

Correspondence: Angela Marino, Department of Human and Social Sciences, University of Messina, viale Ferdinando Stagno D'Alcontres 31, 98166 Messina, Italy.
Tel: +39.090.6765214 - Fax: +39.090.394030.
E-mail: marinoa@unime.it

Journal of Biological Research 2015; 88:5161

Reverse phase protein microarray technology to provide new diagnostic markers of metabolism in rare diseases

F. Santacatterina,[1-3] C. Navarro,[2,4] M. Sánchez-Arago,[1-3] M.A. Martin,[2,3] J.M. Cuezva[1-3]

[1]Department of Molecular Biology, Center of Molecular Biology Severo Ochoa CSIC-UAM, Autonomous University of Madrid; [2]CIBERER-ISCIII, Madrid; [3]Institute of Hospital Investigation, Madrid; [4]Department of Pathology and Neuropathology, University Hospital of Vigo, Spain

Energy metabolism offers a valuable gauge to monitor the genetic alterations that promote cellular dysfunction and hence, is a useful biomarker in human Rare Diseases.

Genetic alterations that result in cellular dysfunction and thus in an overt phenotypic presentation are usually accompanied by alterations in the proteome of energy metabolism. Cancer provides one example.[1] The development of high-throughput OMIC techniques allows the simultaneous interrogation of a large number of genes, proteins and metabolites in the same assay. Reverse phase protein microarrays (RPPmA) is a high-throughput proteomic technique that allows the quantification (fentomolar range) of a given marker in minute amounts of protein from biological specimens. The application of this technique in oncology has been largely documented as it is most useful for the identification and quantification of biomarkers of survival and of the response to chemotherapy. Herein, we have studied the expression of twenty proteins of energy metabolism which include members of the TCA cycle, β-oxidation, electron transport, oxidative phosphorylation, glycolysis and oxidative stress using highly specific antibodies in a cohort of seventy three muscle biopsies of control donors and patients affected of neuromuscular diseases. The cohort included Duchenne (DMD), Becker (DMB), symptomatic forms of DMD and DMB in female carriers (Xp21 Carriers) and Limb Girdle Muscular Dystrophy Type 2C (LGMD2C) biopsies as well as of patients affected of glycogenesis type V (Mc Ardle disease), complex I mitochondrial myopathies, various intensive care unit myopathies (ICU) and neuronal ceroid lipofuscinosis (NCL) also known as Batten disease, a neurodegenerative disease. The samples were obtained with informed consent following the Declaration of Helsinki and coded for anonymity. The final aim of the study was to verify the potential applicability of RPPmA technique in the field of Rare Diseases for the identification of new molecular markers of diagnosis to contribute to the improvement of the clinical handling of these patients. The results indicate that the phenotype of energy metabolism offers relevant diagnostic markers in Rare Diseases.

Reference

1. Aldea M, Clofent J, Núñez de Arenas C, et al. Reverse phase protein microarrays quantify and validate the bioenergetic signature as biomarker in colorectal cancer. Cancer Letters 2011;311:210-8.

Correspondence: Fulvio Santacatterina, Department of Molecular Biology, Center of Molecular Biology Severo Ochoa CSIC- UAM, Autonomous University of Madrid, calle Caracas 8, 28010 Madrid, Spain.
E-mail: fsantacatterina@cbm.uam.es

Nutrition in inflammatory bowel disease patients

A. Abruzzo,[1] G. Tomasello,[2,3] E. Sinagra,[2,4] F. Cappello,[3,5] P. Damiani,[3,4] F. Damiani,[1] M. Bellavia,[3] C. Campanella,[3,6] F. Rappa,[3,6] A. Marino Gammazza,[3,7] L. Cicero,[8] A.I. Lo Monte[1,2]

[1]School in Surgical Biotechnology and Regenerative Medicine, Faculty of Medicine, University of Palermo; [2]Department of Surgical, Oncological and Stomatological Sciences, University of Palermo; [3]S. Raffaele-G. Giglio Institute Foundation, Cefalù; [4]Department of Experimental Biomedicine and Clinical Neuroscience, Section of Human Anatomy, University of Palermo; [5]School of Medicine Specialization in Geriatrics, University of Palermo; [6]Euro-Mediterranean Institute of Science and Technology, Palermo; [7]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [8]Institute for Experimental Veterinary Medicine of Sicily A. Mirri, Palermo, Italy

Inflammatory bowel disease (IBD) is a chronic disorder characterized by a relapsing-remitting course, which alternates between active and quiescent states, ultimately impairing a patients' quality of life.

The two main types of IBD are Crohn's disease (CD) and ulcerative colitis (UC). CD shows a transmural granulomatous inflammation that can involve any segment of the intestine affecting all layers of the intestinal wall while UC is limited to the mucosa and superficial submucosa of the colon. In physiological conditions the gut is costantly exposed to various antigens, commensal microflora and pathogens and the inflammatory response is finely balanced. Anyhow in some individuals with genetic susceptibility an anomalous inflammatory response can arise due to the deregulation of the negative feedback mechanisms implicated in its self-regulation. It is thought that a vast number of environmental risk factors may be implicated in the development of IBD, including smoking, dietary factors, psychological stress, use of non- steroidal anti-inflammatory drugs and oral contraceptives, appendectomy, breastfeeding, as well as infections. Nutritional support as a primary therapy has a crucial role in the management of patients with IBD since it can control the inflammatory process, treat malnutrition and its consequences, and avoid the use of immune-modulating drugs and their side effects. The gut microbiota is clearly manipulated by dietary components such as n-3 PUFA and coniugated linoleic acid (CLA) which favorably reduce endotoxin load via shifts in the composition and metabolic activity of the microbial community.

In particular, the beneficial effect of n-3 polyunsaturated fatty acids (PUFAs) and fermentable fiber, during the remission/quiescent phase of both CD and UC is highlighted. In fact, PUFAs are associated with a less grade of inflammation since they are metabolized to 3-series prostaglandines and thromboxanes and 5-series leukotrienes and, in addition, exert antiinflammatory effects when compared with their n-6 PUFA counterparts. In similar action to dietary n-3 PUFA, coniugated linoleic acid (CLA) have been reported to ameliorate intestinal inflammation in animal models of IBD. In contrast to corticosteroids, CLA suppresses gut inflammatory responses while enhancing antigen specific responsiveness of T cells against viral and bacterial pathogens.

Available data about nutritional interventions do not always match due to the incomplete knowledge of pathogenic mechanisms underlying IBD development. Further studies are therefore needed to improve nutritional therapeutic approach. In particular, is still unclear the role of the fiber in helping the remission of the disease. There are mainly two theories. On one hand, dietary fibers can act as effective prebiotics by altering the intestinal microbial composition and promoting the growth of beneficial bacterial communities within the large intestine.

Some authors reported a positive effect associated with the production by colonic microflora of short chain fatty acids (SCFA), able to down-regulate the production of pro-inflammatory cytokines, to promote the restoration of intracellular Reactive Oxygen Specie (ROS) balance, and the activation of NF-kB.

On the other hand, fibers can promote diarrhea, pain and gas aggravating the clinical state. We suggest that the consumption of fermentable fibers may have a good impact on patients' health. Now is well known that various SNPs are linked to the risk of IBD development and therefore there is the possibility of predict if an individual is predisposed to the disease. The identification of some polymorphisms has an essential role because it allows the modification of diet in the hope of controlling symptoms or preventing relapse. As a consequence, foods that can potentially exacerbate symptoms are eliminated and substituted with those that promote a well-being state.

Correspondence: Giovanni Tomasello, Department of Surgical, Oncological and Stomatological Sciences, University of Palermo, Via Liborio Giuffrè 5, 90127 Palermo, Italy.
E-mail: giovanni.tomasello@unipa.it

Journal of Biological Research 2015; 88:5161

Tecniche di defogliazione applicate a vitigni e vini di Nero d'Avola per la determinazione delle sostanze nutraceutiche

P. Agozzino,[1] G. Avellone,[1] F. Filizzola[2]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [2]Research and Scientific Development Area, University of Palermo, Italy

Introduzione

Studi condotti sui vini rossi, hanno dimostrato come un loro moderato consumo quotidiano possa ridurre l'incidenza delle malattie cardiovascolari[1] perché in essi sono contenute sostanze antiossidanti ed in particolare il transresveratrolo e il piceatannolo.[2] I meccanismi protettivi sarebbero indipendenti dall'effetto di quote equivalenti di alcool contenute nel vino ma riconducibili alle caratteristiche del vitigno e al contenuto di polifenoli, le cui concentrazioni dipendono molto dalla tecnica di vinificazione (flavonoidi, trans-resveratrolo e tannini polimerici).[3] Con l'obiettivo di migliorare la quantità di polifenoli in una cultivar di Nero d'Avola, e stata effettuata su alcuni filari di un Vigneto sito presso Castelbuono (PA), la tecnica di defogliazione eseguita alla fase fenologica di acino pisello su un campione di 500 piante con l'eliminazione manuale delle foglie fino al primo nodo sopra il grappolo. I confronti sono stati eseguiti con un testimone non defogliato (controllo).

Materiali e Metodi

Il contenuto in solidi solubili (°Brix) è stato determinato con un rifrattometro di Abbe. Il pH è stato misurato con piaccametro. Per la determinazione degli antociani totali, le bucce degli acini sono state poste in un tampone tartarico a pH 3,2 al fine di estrarre tutti i polifenoli, l'acidificazione del campione è stata eseguita con HCl conc., la diluizione con etanolo cloridrico, l'analisi spettrofotometrica (UV) misurando l'assorbanza a 540 nm utilizzando cuvette in quarzo con cammino ottico di 1 cm. Per la determinazione dei flavonoidi totali si misura la densità ottica a 280 nm e l'assorbanza dipendendo dalla concentrazione. La determinazione delle proantocianidine viene fatta per differenza fra l'assorbanza a 540 nm, misurata prima e dopo l'idrolisi e confronto del Δ di assorbanza con una curva di calibrazione determinata con soluzioni standard di proantocianidine. Lo studio della componente polifenolica è stato eseguito su campioni di bucce e polpa, acino e raspo, rachide e vino. Le analisi sono state condotte con tecnica HPLC con cromatografo liquido Agilent 1100, rilevatore a serie di diodi (DAD) posizionato a 305 e 325 nm, autoiniettore con volume di iniezione 50 μl, colonna Phenomenex Luna C18, eluenti: acetonitrile e tampone fosfato (KH2PO4+H3PO4) 0,02 M a pH=3,0, flusso 1,0 ml/min., per calcolare i tempi di ritenzione sono stati preventivamente iniettati i rispettivi standard. Buccia e polpa, acino intero e raspo, rachide hanno subito lo stesso trattamento: macerazione con CH3OH al 95%, omogeneizzazione e agitazione tramite agitatore orbitale, filtrazione con filtro in microfibra di vetro, evaporazione in rotovapor, prima estrazione con NaHCO3 al 5% seguita da tre estrazioni con acetato di etile, evaporazione dell'estratto e risospensione con CH3OH 80%. Sul vino sono state effettuate le analisi in HLPC senza trattamenti preliminari.

Risultati e Discussione

Gli obiettivi dello studio sono stati, analizzare l'andamento dei componenti dell'uva nelle differenti tesi e valutare l'influenza dei fattori studiati sul biochimismo dei polifenoli.

Dalla determinazione nel vino del contenuto di antociani totali (controllo=201 mg/L, defogliato=267), flavonoidi totali (controllo=1832 mg/L, defogliato=2122) e proantocianidine (controllo=1761 mg/L, defogliato=1767), si deduce che l'aumento di esposizione degli acini nelle prime fasi di sviluppo ha prodotto un incremento degli antociani e degli altri flavonoidi. La defogliazione ha però causato uno squilibrio nelle dinamiche di maturazione, maturità fenolica raggiunta prima di quella tecnologica. La tesi controllo si è comportata in maniera più equilibrata presentando dinamiche di maturazione più regolari.

Dai parametri di altre analisi riportati in Tabella 1 si evince che la tecnica della defogliazione non ha comportato un aumento di antociani, flavonoidi e prontocianidine nelle bucce.

Dall'analisi della componente di alcuni polifenoli (Tabella 2) si può notare che il trattamento di defogliazione effettuato ha comportato un aumento delle concentrazioni dei composti piceatannolo glicosilato, trans-resveratrolo glicosilato e piceatannolo negli acini di grappoli provenienti da viti defogliate mentre nel vino i valori relativi agli stessi composti nelle le due tesi sono comparabili. Per quanto riguarda il trans-resverratrolo, la sua concentrazione è maggiore nella tesi controllo.

Correspondence: Pasquale Agozzino, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Viale delle Scienze 16, 90128 Palermo, Italy.
Tel: +39.091.23891201.
E-mail: pasquale.agozzino@unipa.it

Tabella 1. La scala in gradi Brix mostra la concentrazione percentuale di tutte le sostanze disciolte nell'acqua. L'indice degli antociani totali (mg/acino) esprime il contenuto di antociani monomeri e polimeri. L'indice dei flavonoidi totali (mg/acino) esprime il contenuto di antociani e tannini.

	16 Agosto		24 agosto		19 settembre	
Tesi	Controllo	Defogliato	Controllo	Defogliato	Controllo	Defogliato
Solidi solubili (°Brix)	21,00	21,60	24,35	24,51	24,10	24,51
pH	2,89	2,95	2,98	2,94	3,43	3,37
Acidità titolabile (g/L ac. tartarico)	13,30	10,70	11,70	10,00	8,10	8,40
Alcool (% V)	12,20	12,65	14,55	14,70	14,40	14,70
Antociani totali nelle bucce (mg/acino)	0,93	1,44	1,47	1,82	1,62	1,51
Flavonoidi totali nelle bucce (mg/acino)	2,75	3,54	3,48	4,10	4,10	3,98
Prontocianidine totali nelle bucce (mg/acino)	2,23	2,64	2,89	2,39	2,06	2,66

Tabella 2. Analisi della componente polifenolica.

Composto	Vino controllo	Vino defogliato	Polpa e buccia controllo	Polpa e buccia defogliato	Acini e raspo controllo	Acini e raspo defogliato	Rachide controllo	Rachide defogliato
Piceatannolo glicosilato	0,44	0,45	78,39	93,58	0,21	0,34	13,72	10,74
Trans-resveratrolo glicosilato	0,16	0,15	39,37	55,92	46,60	51,52	57,13	40,51
Piceatannolo	0,17	0,15	3,58	5,48	3,045	4,19	91,32	12,53
Trans-resveratrolo	0,20	0,13	0,130	0,03	0,04	nd	0,13	0,15

Concentrazione espressa in ng/µL.

Conclusioni

Le analisi condotte per la determinazione dei flavonoidi hanno evidenziato che il controllo ha presentato dinamiche di maturazione più regolari. Ciononostante, il vino della tesi defogliata ha presentato un maggior contenuto di antociani. Le analisi condotte sugli stilbeni hanno evidenziato che i valori del piceatannolo glic., trans-resveratrolo glic. e piceatannolo sono confrontabili nel vino (defogliato e controllo), mentre si trovano in maggiore concentrazione nel grappolo defogliato. Il trans-resveratrolo è invece presente in maggiore quantità nel controllo. La defogliazione influisce più sull'uva rispetto al vino. In base ai dati ottenuti, considerando le condizioni pedo-climatiche del luogo dove è stata eseguita la sperimentazione, la defogliazione risulta essere una operazione non necessaria, non influenzando la componente polifenolica del prodotto finale.

Bibliografia

1. Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 1992;339:1523-6.
2. Careri M, Corradini C, Elviri L, et al. Liquid chromatography-electrospray-tandem mass spectrometry of cis-resveratrol and trans-resveratrol: development, validation and application of the method to red wine, grape and winemaking by products. J Agric Food Chem 2004;52:6868-74.
3. Kondo K, Matsumoto A, Kurata H, et al. Inhibition of oxidation of low-density lipoprotein with red wine. (letter) Lancet 1994;344.

A 3D totally absorbable synthetic mesh in antireflux surgery: Gore Bio-A tissue reinforcement for hiatal hernia repairing

A. Agrusa, G. Romano, G. Frazzetta, A. Inviati, G. De Vita, S. Di Giovanni, D. Chianetta, G. Di Buono, S. Bonventre, G. Gulotta

Department of General and Emergency Surgery, University of Palermo, Italy

Introduction

Hiatal hernia, defined as *transitory or stable dislocation of a part of the stomach in mediastinum through the diaphragmatic crura delimiting esophageal hiatus*. Its appearance presupposes anatomic anomalies or weakening of structures and mechanisms able to maintain esophago- gastric junction and stomach in the abdominal cavity.[1] Classically hiatal hernia was classified in four types using Hill's classification: Type 1 hiatal hernia is associated with GERD in 50-90% of cases, in facts its presence gradually compromises esophago-gastric junction's continence favouriting the backwater of acid secretion and its reflux in contact with esophageal mucosa during transient relaxations of the LES and also reducing clearing systems overall for large hiatal hernias.[2,3] Several randomized controlled trials with long-term follow-up comparing surgical with medical therapy for the treatment of GERD, strongly support surgery as an effective alternative to medical therapy.[4] Fundoplication has also been demonstrated to lead to improved or at least comparable quality of life to that of medically treated patients and it is associated with high patients satisfactions rate.[5] A laparoscopic total fundoplication is considered today the procedure of choice increasing the resting pressure and length of the lower esophageal sphincter, decreasing the number of transient LES relaxations and improving quality of esophageal peristalsis and follow-up demonstrates complete symptoms control in 80-90% of patients 10 years later.[6] However primary laparoscopic hiatal hernia repair is associated with up 42% recurrence rate.[7] Several level data suggest that mesh reinforcement of the crural closure for hiatal hernia repair decreases the recurrence of hernia, but can lead to esophageal erosion and stenosis or disphagya, above all non-absorbable mesh.[8,9] For this clinical case, we experiment a new totally absorbable Gore Bio-A® mesh.[10]

Correspondence: Giorgio Romano, Department of General and Emergency Surgery, University of Palermo, Via L. Giuffrè 5, 90128 Palermo, Italy.
E-mail: giorgio.romano@unipa.it

Journal of Biological Research 2015; 88:5161

Clinical Case

Female patient; 65-year old; 6-year classic history of GERD (regurgitation, belching, bloating, *acid in the throat* treated for several years by multiple proton pump inhibitors); BMI 22. An EGDS revealed a >3 cm hiatal hernia, grade B Los Angeles esophagitis. 24-hour pH study was positive for acid reflux and esophageal manometry revealed LES intrathoracic dislocation. With laparoscopic 5-trocars approach, the hiatal hernia defect was identified and primarily repaired, by crural closure, with size 0 permanent suture (ETHIBOND). GORE BIO-A® Tissue Reinforcement was trimmed to fit the defect with a *U* shape cutout to accommodate the esophagus. It was secured using two absorbable sutures (VICYRL). At least A Nissen fundoplication was performed without incident. Result: Gore BIO-A® mesh was easily placed through a 10-12 mm trocar. It had good handling characteristics laparoscopically, and no pre- operative preparation was required of the prosthetic. It can be cut and tailored intraoperatively to an optimal adaptation. There were no short-term complications from the mesh. The patient had not significant post-operative sequelae.

Conclusions

Crural closure reinforcement during hiatal hernia repair can be done readily with this new totally absorbable Gore Bio A Tissue Reinforcement: it is a 3D web of completely absorbable synthetic polymers replaced by soft tissue over six months; it is a mix of glycolic acid and trimethylene carbonate and its function consistes in stimulating collagens deposition and ingrowth of new connective soft tissue.[11] It was demonstrated that Gore Bio-A increases cellular in-growth in 7-30 days more and more previously than biologics mesh; it also increases new blood vessels formation in 7-14 days reaching the greatest vascular in-growth. Instead the biologic meshes gore BIO-A seems to induce the least inflammatory infiltrate. Gore BIO-A tissue reinforcement seems to have all the best characteristics to hernia hiatal laparoscopic repair reducing both recurrence rates and post-operative mesh-related complications, even if several other cases and studies are necessary. However further data and studies are needed to evaluate long-term efficacy and complications associated with its use.

References

1. Schmidt E, Shaligram A, Reynoso Jf, et al. Hiatal hernia repair with biologic mesh reinforcement reduces recurrence rate in small hiatal hernias. Dis Esophagus 2014;27:13-7.

2. Obeid N, Velanovich V. The choice of primary repair or mesh repair for paraesophageal hernia: a decision analysis based on utility scores. Ann Surg 2013;257:655-64.
3. Braghetto MI, Korn BO, Cardemil HG, et al. Laparoscopic surgery for benign esophageal diseases. Rev Med Chil 2012;140:703-12.
4. Grubnik V, Malinovskiĭ A Analysis of long-term results of laparoscopic repair of hiatal hernias and Nissen fundoplication. Klin Khir 2012;10:34-6.
5. Hermann J, Kościński T, Malinger S, et al. Strangulation of the stomach and the transverse colon following laparoscopic esophageal hiatal hernia repair. Wideochir Inne Tech Malo Inwazyjne 2012;7:311-4.
6. Brandalise A, Aranha N, Brandalise N. The polypropylene mesh in the laparoscopic repair of large hiatal hernias: technical aspects. Arq Bras Cir Dig 2012;25:224-8.
7. Porziella V, Cesario A, Lococo F, et al. Complete transmural gastric migration of ptfe mesh after surgery for a recurrent hiatal hernia. Eur Rev Med Pharmacol Sci 2012;16:42-3.
8. Carlson M, Richards C, Frantzides C. Laparoscopic prosthetic reinforcement of hiatal herniorrhaphy. Dig Surg 1999;16:407-10.
9. Frantzides C, Carlson M. Prosthetic reinforcement of posterior cruroplasty during laparoscopic hiatal herniorrhaphy. Surg Endosc 1997;11:769-71.
10. Masullo JM, Singh TP, Dunnican WJ, Binetti BR. Preliminary study of hiatal hernia repair using polyglicolic acid: trimetilene carbonate mesh. Jlss 2012; 16:55-9.
11. Sutton P, Evans J, Uzair S, Varghese J. The use of gore bio-a in the management of the open abdomen. Bmj Case Rep 2013; 25:2013.

Synthesis and structure-activity relationship studies of new 3-methyl-5-(5-propyl-1*H*-1-R'-3- pyrazolyl)-1*H*-1-R-4-nitrosopyrazoles as antimicotic agents

S. Aiello,[1] L.C. López-Cara,[2] F. Venturella,[3] A. Licata[4]

[1]Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, Italy; [2]Department of Pharmaceutical and Organic Chemistry, University of Granada, Spain; [3]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [4]Gastroenterology Unit, Department of Internal and Specialized Medicine, University of Palermo, Italy

Many ubiquitous yeast are primarily pathogens for immunocompromised patients, individuals with AIDS and organ transplanted are at high risk of cryptococcosis and candidiasis. In this setting, fungal infections are particularly difficult to treat because antifungal therapy usually does not eradicate the infection and require lifelong treatment with antifungal drugs. Consequently, the need for novel antifungal agents for opportunistic infections is apparent in light of significant problems associated with current drugs and makes the development of new drug entities all more urgent.

We have reported that some 3-(3-alkyl-4-nitroso-1*H*-5-pyrazolyl)-5-R-isoxazoles[1] and the isomeric 5-(1-alkyl-4-nitroso-1*H*-3-pyrazolyl)-3-R-isoxazoles[2] showed *in vitro* potent antifungal activity at non cytotoxic concentrations. This antifungal activity was correlated to: i) the interaction of the isoxazolic nitrogen with the alkyl group bound to the pyrazolyl nitrogen; ii) the *cis* or *trans* configuration adopted by the nitroso group with respect to the alkyl chain bound to the pyrazolic nitrogen and perpendicularly folded to the molecular plane.

To verify this hypothesis, we synthesized compounds in which the isoxazole was substituted by a pyrazole moiety, leading to the new isomeric series I and II (Figure 1). The title compounds tested *in vitro* for antifungal activity against *C. Neoformans* and *C. Krusei*, displayed an interesting antifungal activity, in particular compound Ib was 2 and 32 fold more potent than Amphotericin B and Fluconazole, respectively, against *C. krusei,* fungus with intrinsic resistance to many of antifungal azoles.

These results suggest that, depending on the heterocyclic molecule bound to the 5 position of 1*H*-1-R-4-nitrosopyrazoles, it is possible to modulate the antifungal activity of 4- nitrosopyrazoles. *In vitro* metabolism studies and *in vivo* assay are in progress for all described compounds.

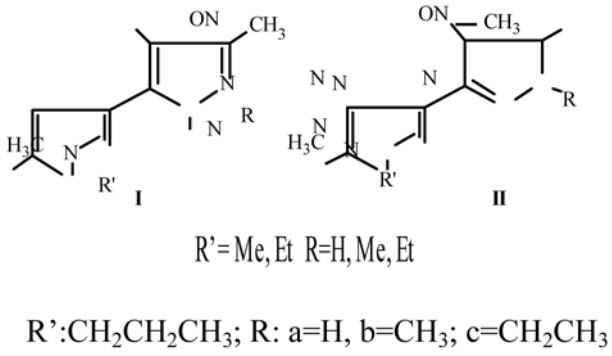

Figure 1. Isomeric series I and II.

References

1. Aiello E, Aiello S, Mingoia F, et al. Synthesis and antimicrobial activity of new 3-(1-R-3- methyl-4-nitroso-1*H*-5-pyrazolyl)-5-methylisoxazole derivatives. Bioorg Med Chem 2000;8:2719-28.
2. Aiello S, Aiello E, Orioli M, Carini M. 3-(1-R-3-methyl-4-nitroso-1H-5-pyrazolyl)-5-methylisoxazoles: a new class of antifungal compounds. *In vitro* metabolism by rat liver:LC and LC-MS studies. Convegno Nazionale SCI, Sorrento 18-22 Settembre 2002.

Correspondence: Stefania Aiello, Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, via Caduti senza Croce 3, 90127 Palermo, Italy.
E-mail: stefania.aiello@unipa.it

Journal of Biological Research 2015; 88:5161

Distinct biological effects are observed in HT-29 colorectal carcinoma cells induced to express K-RASG12V or K-RASG13D

M.M. Barreca, M.R. Saladino, I. Albanese

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

RAS are small membrane-bound GTPase proteins involved in signalling pathways that regulate proliferation, differentiation and apoptosis in all cell types, and whose activating mutations have oncogenic effects. The three major isoforms of p21 RAS (H-, K- and N-RAS) have a high degree of homology, especially in the regions involved in interactions with GDP/GTP and with regulatory proteins and effectors, but differ significantly in the C-terminal

25 amino acids region. This hypervariable domain is the site of post-translational modifications specific for each isoform, which result in distinct intracellular trafficking routes and final subcellular localizations, where the type and concentration of regulators and effectors may differ.[1] This may explain the observed non-overlapping functions of these proteins, the different biological effects of their physiological activation, and their differential involvment, when mutated, in specific tumor types. In almost all cases, the genetic alterations detected in tumoral cells are missense point mutations in codons 12 or 13, more rarely in codon 61, and they always result in a constitutively active protein by inactivating its GTPase activity. Mutations in the K- RAS isoform are a frequent, early event in colorectal tumorigenesis and their occurrence is considered to be a resistance factor to therapies based on anti-EGFR monoclonal antibodies.[2]

However, molecular epidemiological studies in different primary and metastatic tumors suggest that mutations in different codons or different mutations in the same codon of Ras may have diverse biological consequences[3] and may lead to a different response to drug treatments. In particular, one of the new drugs developed for the treatment of colorectal carcinoma is Cetuximab, a monoclonal chimeric human/mouse antibody IgG1, which acts against the extracellular domain of EGFR. The binding of this antibody to the receptor causes a direct inhibition of its tyrosine kinase activity resulting in the inhibition of several pathways of signal transduction mediated by RAS, such as those of PI3K/AKT, and RAF/MAPKs. This stimulates pro-apoptotic mechanisms and the inhibition of cell proliferation. Several clinical trials conducted in recent years have shown that patients with CRC who have mutations in K-ras codon 12 or 13 respond heterogeneously to Cetuximab treatment and for this reason are currently excluded from treatment with this drug. However, it has recently been reported that tumors bearing the K-RASG13D mutation may show some response to the therapy.[4] It is also currently unclear whether mutations in BRAF (an effector of RAS) affect the response to Cetuximab.[2]

To shed more light on the molecular mechanisms responsible for the different effects of Ras mutations, we established an experimental system by isolating stable clones of HT-29 cells (a human colorectal adenocarcinoma cell line characterized by mutations in the BRAF and PIK3CA genes and in which the endogenous Ras genes are wild type) transfected with cDNAs codifying K-RASG12V (clone K12) and K-RASG13D (clone K13) under the control of a Mifepristone-inducible promoter. Cell proliferation assays and cytofluorimetric analysis reveal that activation of the expression of K-RASG12V and of K-RASG13D have distinct biological effects on the cells. We have also analysed the response of the induced and not induced cells to treatment with inhibitors of the two main RAS effectors (MEK and PI3K) and with the anti-EGFR monoclonal antibody Cetuximab.

Correspondence: Ida Albanese, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze 16, 90128 Palermo, Italy.
E-mail: ida.albanese@unipa.it

Journal of Biological Research 2015; 88:5161

References

1. Priora IA, Hancockb JF. Ras trafficking, localization and compartmentalized signalling. Seminars in Cell & Developmental Biology 2012;23:145-53.
2. Grossmann AH, Samowitz WS. Epidermal growth factor receptor pathway mutations and colorectal cancer therapy. Arch Pathol Lab Med 2011;135:1278-88.
3. Andreyev HJN, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. Journal of the National Cancer Institute 1998;90:675-84.
4. De Roock W, Jonker DJ, Di Nicolantonio F, et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA 2010;304:1812-20.

Valutazione degli effetti antiossidanti del *Resolvis Omega*™ in un modello di disfunzione corneale *in vitro*

L. Di Rosa,[1] M. Allegra,[1] D. Aleo,[2] A. Attanzio,[1] L. Tesoriere,[1] C. Gentile,[1] M.G. Saita,[2] M.A. Livrea[1]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [2]Medivis Catania, Italy

EPA (acido eicosapentaenoico) e DHA (acido docosaesaenoico) sono derivati metabolici degli AGE (acidi grassi essenziali) e in particolare dell'acido linolenico (omega-3). Gli acidi grassi omega-3 (DHA ed EPA) sono considerati AGE perché il nostro organismo non è in grado di sintetizzarli e il loro apporto è possibile solo attraverso l'assunzione di alimenti che li contengono. Gli acidi grassi omega-3 sono molecole antinfiammatorie e come tali sono state associate ad una riduzione del rischio cardiovascolare e delle patologie correlate ad una inappropriata attivazione del sistema immunitario. Grazie alle loro caratteristiche chimico-fisiche contribuiscono a mantenere la *flessibilità* delle pareti dei vasi arteriosi, delle membrane cellulari e a ridurre i livelli di lipidi nel sangue. Infine, un aumento della loro concentrazione nei tessuti è stato associato ad una inibizione dei processi biochimici associati alla senescenza e alle patologie oculari su base infiammatoria e degenerativa.[1,2]

Alla luce delle ormai note interconnessioni tra le reazioni infiammatorie e lo stress ossidativo, il presente studio ha valutato le proprietà antiossidanti del *Resolvis Omega*™, un nuovo farmaco a base di EPA/DHA in un modello sperimentale di disfunzione di cellule corneali umane (HCE) da perossido d'idrogeno, *in vitro*.

Da un punto di vista sperimentale, gli effetti antiossidanti sono stati analizzati, tramite analisi citofluorimetrica delle specie reattive di ossigeno e azoto intracellulari.[3] I nostri risultati dimostrano che HCE stimolate con H_2O_2 200 μM producono ROS intracellulari con una cinetica che raggiunge il picco massimo a 5 ore per poi raggiungere i livelli controllo entro 8 ore. Durante questo intervallo temporale, il pretrattamento delle HCE per 1h con *Resolvis Omega*™ determina una significativa riduzione dei ROS endocellulari a 3, 4, 5, e 6 ore ($P<0.05$) (Figura 1).

Il trattamento con il solo veicolo, in assenza di H_2O_2 e il pretrattamento con il veicolo in assenza di *Resolvis Omega*™, non determina alcun aumento o diminuzione dei livelli di ROS endocellulari, durante l'intervallo di tempo di osservazione (dati non mostrati).

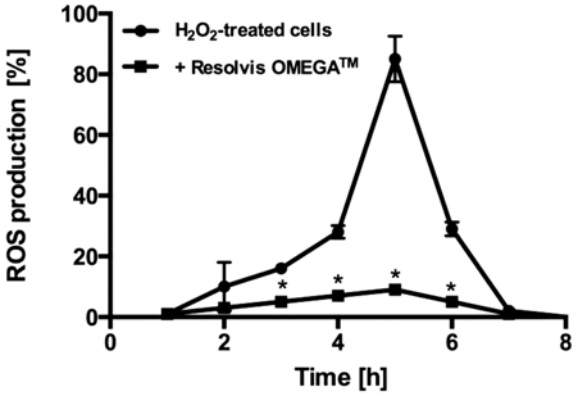

Figura 1. Valutazione degli effetti del *Resolvis Omega*™ sulla cinetica di produzione di ROS in HCE attivate da H_2O_2. Le cellule sono state stimolate con H_2O_2 (200 μM) in un intervallo temporale compreso tra 0 e 8 h, in assenza e in presenza di *Resolvis Omega*. I valori sono la media±SE di 3 esperimenti separati condotti in triplicato.

Questi risultati dimostrano che *Resolvis Omega*™ alle concentrazioni usate comunemente nella pratica oculistica, esercita dei significativi effetti antiossidanti e costituisce un solido *rationale* per una ulteriore investigazione delle proprietà antiinfiammatorie di questo farmaco nelle patologie oculari.

Bibliografia

1. Jump DB, Depner CM, Tripathy S. Omega-3 fatty acid supplementation and cardiovascular disease. J Lipid Res 2012;53:2525-45.
2. Fetterman JW Jr., Zdanowicz MM. Therapeutic potential of n-3 polyunsaturated fatty acids in disease. Am J Health Syst Pharm. 2009;66:1169-79.
3. Allegra M, D'Acquisto F, Tesoriere L, et al. Cross-talk between minimally primed HL-60 cells and resting HUVEC reveals a crucial role for adhesion over extracellularly released oxidants. Biochem Pharmacol 1;81:396-401.

Correspondence: Mario Allegra, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Viale delle Scienze 16, 90128 Palermo, Italy.
Tel: +39.091.23891201.
E-mail: mario.allegra@unipa.it

Journal of Biological Research 2015; 88:5161

Carbohydrate digestibility on wheat durum bread: preliminary hypotheses on raw materials role

N. Amato,[1] S. Lombardo,[2] G. Pandino,[2] G. Russo[3]

[1]Department of Agricultural, Food and Environment Sciences, University of Foggia; [2]Department of Agricultural and Food Production Sciences, University of Catania; [3]Reserach Consortium G.P. Ballatore, Palermo, Italy

The 1997 FAO/WHO Expert Consultation suggested that the Glycaemic Index (GI) might provide an useful help to select the most appropriate carbohydrate-containing foods in order to reduce several diseases. The glycaemic response to food, which in turn affects the insulin response, depends on the rate of gastric emptying, digestion and absorption of carbohydrate from the small intestine, as well as on the effects of the other food factors to potentiate non-glucose mediated insulin secretion. A range of food factors has been identified as important determinant of the glycaemic response to carbohydrate foods. Therefore, different food products or composition of meals with the same amount and type of carbohydrates show differences in glycaemic and insulinemic responses.

In this view, the aim of this research was to identify the technological factors which influence the glycaemic index (GI) in bread making of reground semolina and wholemeal flour. Five ancient accessions (Russello, Timilia, Bidì, Biancolilla and Senatore Cappelli) were compared to five modern commercial blends and an ancient mix between Timilia and Russello accessions under technological and nutritional profiles. The parameters under study were: moisture, protein, total starch, ash, farinographic profile, wet and dry gluten, gluten index, -amylase activity, particle size and damaged starch.

Our results showed the best attitude of wholemeal flour by old accessions in a health point of view, due to the higher gluten content, poor quality of gluten and greater absorption of water. On the contrary, modern varieties of reground semolina are more suitable for breadmaking, owing to the higher technological attitude and the greater alpha-amylase activity.

This work is considered the first step of a PhD project conducted within the University of Foggia.

Correspondence: Nathascia Amato, Department of Agricultural, Food and Environment Sciences, University of Foggia, via Napoli 25, 71100 Foggia, Italy.
E-mail: nathascia.amato@unifg.it

Journal of Biological Research 2015; 88:5161

Anticancer drug delivery system based on vaterite particles

B. Parakhonskiy,[1,2] Y. Svenskaya,[1,3] A. Haase,[4] E. Lukyanets,[5] R. Antolini[4]

[1]BIOtech Center Department of Industrial Engineering, University of Trento, Mattarello, Italy; [2]AV Shubnikov Institute of Crystallography, Russian Academy of Science, Moscow, Russia; [3]Saratov State University, Russia; [4]Department of Physics, University of Trento, Italy; [5]Organic Intermediates and Dyes Institute, Moscow, Russia

Photodynamic therapy (PDT) is a promising therapeutic option in the management of cancer and other diseases. The mechanism of photodynamic therapy is based on the selective combination of non-toxic dyes or photosensitizers with harmless visible light which, in the presence of oxygen, leads to the generation of cytotoxic species and consequently to cell apoptotic or necrotic death.[1] Since in clinical PDT it is common practice to irradiate wider fields that include healthy tissues which might contain microscopic malignant foci, the photosensitizer selectivity (target versus healthy tissue) become crucial. Otherwise a high concentration of photosensitizer is required to ensure enough concentration at the tumor site, with consequent incidental toxicity in healthy tissue and increased treatment cost. Both side effects and treatment costs could be significantly reduced by targeted delivery of the photosensitizer to the region of interest at a well-defined time.

In this work we use *Photosens* (a mixture of sulfonated aluminum phthalocyanines $AlPcS_n$, with n=2, 3 or 4) as photosensitizing drug[2] and calcium carbonate ($CaCO_3$) as delivery system. $CaCO_3$ exists in three different anhydrous crystalline polymorphs: calcite, aragonite, and vaterite. Vaterite particles have a large porosity, large surface area, and can decompose fast under relatively mild conditions. Vaterite is the least stable phase of $CaCO_3$ since it slowly dissolves and recrystallizes to form calcite in contact with water. Previous studies described the possibility of synthesizing spherical mono-dispersed vaterite particles in the size range from 2 to 10 μm[3] and could very recently be downscaled to 400 nm[4] which will strongly improve the cellular uptake efficiency. A controllable release mechanism based on a crystal phase transition has recently been demonstrated.[5,6] Preliminary tests have been performed to assess the particles cell toxicity, cell uptake and drug release.[5] Also, as a proof of principle, the pH sensitivity of the delivery system has been demonstrated,[7] as well as the possibility to be used as a sensor platform.[8]

The release mechanism depending on the surrounding pH has been studied, showing a fast degradation of the carriers in buffers below pH 7. These results hold out the prospect of a novel photodynamic therapy drug delivery system. Variations of particle size or additional coatings allow to custom-design workload release curves. An intrinsic cancer-sensitivity can be expected from the pH-dependent release in the acidic microenvironment of cancer tissue.

During our work, we performed a detailed study of the cumulative release and recrystallization process in different media and in the pH range from 3 to 7. Modification of particle size and pH will allow customizing the workload release curves. A drug delivery system with pH-controlled release promises intracellular delivery with a high selectivity to cancer cells. In combination with an otherwise poorly selective photosensitizer, this could become a strong cancer-therapeutic tool, where the carrier degradability could be tuned to control the rate of drug release. We investigated the encapsulation efficiency for the photosensitizer in micrometer- and sub- micrometer-sized carriers. Release mechanism dependent on the surrounding pH was studied, showing a fast degradation of the carriers in buffers below pH 7.

These results hold out the prospect of a novel drug delivery system. Cancer-sensitivity can be achieved due to the enhanced uptake and fast release in the low pH endocytic vesicles of viable cancer cells.

Correspondence: Renzo Antolini, Department of Physics, University of Trento, via Sommarive 14, 38123 Trento, Italy.
E-mail: renzo.antolini@unitn.it

Journal of Biological Research 2015; 88:5161

References

1. DeRosa M, Crutchley R. Photosensitized singlet oxygen and its applications. Coord Chem Rev 2002;233-234:351-71.
2. EA Lukyanets. Phthalocyanines as photosensitizers in the photodynamic therapy of cancer J Porphyr Phthalocyanines 1999;3:424-32.
3. Schmidt S, Volodkin DV. Microparticulate biomolecules by mild $CaCO_3$ templating. J Mater Chem B 2013;1:1210.
4. Parakhonskiy BV, Haase A, Antolini R. Sub-micrometer vaterite containers: synthesis, substance loading, and release. Angew Chem Int Ed Engl 2012;51:1195-7.
5. Parakhonskiy BV, Foss C, Carletti E, et al. Tailored intracellular delivery *via* a crystal phase transition in 400 nm vaterite particles. Biomater Sci 2013;1:1273-81
6. Parakhonskiy BV, Tessarolo F, Haase A, Antolini R. Dependence of sub-micron vaterite container release properties on pH and ionic strength of the surrounding solution. Adv Sci Technol 2012;86:81-5.
7. Svenskaya Y, Parakhonskiy BV, Haase A, et al. Anticancer drug delivery system based on calcium carbonate particles loaded with a photosensitizer. Biophys Chem 2013;182:11-5.
8. Yashchenok AM, Borisova D, Parakhonskiy BV, et al. Nanoplasmonic smooth silica versus porous calcium carbonate bead biosensors for detection of biomarkers. Ann Phys 2012;524:723-32.

Assessment of three different anticoagulants and storage time influence on haematological parameters in *Mugil Cephalus (Linneaus, 1758)*

C. Faggio,[1] F. Fazio,[2] F. Arfuso,[2] G. Fortino,[2] G. Piccione[2]

[1]Department of Biological and Environmental Sciences, University of Messina; [2]Department of Veterinary Sciences, University of Messina, Italy

Interpretation of fish haematological data is quite difficult due to internal and external variation. It is routinely recommended that haematologic determinations on blood samples are carried out immediately after blood collection, and if not possible, the samples should be refrigerated until determination to minimize artifactual changes.[1] Moreover, as fish blood tends to clot rapidly and clotting becomes faster when it is warm or is under stress condition related to experimental procedures,[2] the use of anticoagulants is necessary to obtain reliable results of blood analyses. The effects of various types of anticoagulants in haematology were studied in various fish species,[3,4] but no information's for *Mugil cephalus* (*M. cephalus*) haematology were found. The aim of the present study was to evaluate the effect of three different anticoagulants and storage time on haematological parameters and differential leukocyte count of *M. cephalus*. Twenty-six adult male mullets (*M. cephalus*), caught from Faro Lake (Sicily, Italy), were immediately subjected to blood sampling from caudal vein. Blood samples were collected in different microtubes containing three different anticoagulants: ethylenediamine tetraacetic acid (EDTA), heparin and sodium citrate respectively. All samples were analyzed immediately (T0) and 24h (+4°C), (T24), after blood collection to assess the follow parameters: red blood cell count (RBC), haematocrit (Hct), haemoglobin (Hb), white blood cell count (WBC), thrombocyte count (TC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC) and manual differential leukocyte count (Lymphocytes, Monocytes, Neutrophils, Eosinophils and Basophils). Mean values±Standard Deviation (SD) of studied haematological parameters and the percentage of leukocyte identification and counting were reported in Table 1. Two-way analysis of variance (ANOVA) for repeated measures showed statistical significant

Table 1. Mean values±standard deviation of haematological parameters and percentage of leukocyte identification and counting obtained in *26 Mugil cephalus*.

Parameters	T0 EDTA	Sodium citrate	Heparin	T24 EDTA	Sodium citrate	Heparin
RBC (x106/µL)	2.55±0.4	2.26±0.8	2.34±0.9	2.30±0.6	2.13±0.6	2.28±0.7
Hct (%)	30.14±5.7[a]	26.89±4.9	25.43±4.8[a]	30.22±5.7	24.17±3.0	26.25±5.8
Hb (g/dL)	7.34±1.1	6.13±1.8[b]	6.54±1.9	7.57±1.0	6.60±1.7[b]	7.27±1.6
WBC (x103/µL)	8.02±2.1	7.95±3.1	8.03±3.3	7.25±2.0	5.08±2.3[c]	7.20±2.9
Lymphocytes (%)	90.8±2.6	90.1±2.6	91.4±3.8	89.8±5.0*	84.4±7.4[c]*	88.3±6.6*
Monocytes (%)	2.5±1.0	2.9±2.1	2.9±2.8	3.3±1.9*	6.0±3.9[c]*	4.0±2.6*
Neutrophils (%)	6.7±2.3	7.0±2.4	5.7±2.3	6.9±3.3	9.2±3.9*	7.3±4.5*
Eosinophils (%)	0.0±0.0	0.0±0.0	0.0±0.2	0.0±0.2	0.1±0.3	0.2±0.5
Basophils (%)	0.0±0.0	0.0±0.0	0.0±0.2	0.0±0.2	0.3±0.3	0.2±0.5
TC (x103/µL)	22.36±3.9[a]	24.59±3.6	24.99±3.1	26.25±3.5[a]*	29.86±2.9*	28.65±3.4*
MCV (fL)	119.84±22.6	130.67±44.2	117.93±32.3	147.95±69.9	122.10±34.9	121.38±37.2
MCH (pg)	28.89±1.8	27.88±5.2	28.99±3.7	38.39±16.5*	33.23±10.7*	34.22±12.5*
MCHC (g/dL)	24.65±2.8	23.19±7.2	25.65±4.9	25.59±4.0*	27.56±7.4*	27.89±3.6*

Significances (anticoagulants): [a]*vs* heparin and sodium citrate at T0; [a1]*vs* heparin and sodium citrate at T24; [b]*vs* EDA at T0; [b1]*vs* EDTA at T24; [c]*vs* heparin and EDTA at T24. Significances (storage time): **vs* T0.

Correspondence: Caterina Faggio, Department of Biological and Environmental Sciences, University of Messina, Via F. Stagno D'Alcontres 31, 98166 Messina, Italy.
E-mail cfaggio@unime.it

Journal of Biological Research 2015; 88:5161

effects of different anticoagulants and storage time for some parameters studied. In particular, statistical analysis showed significant effect of anticoagulants on Hct, Hb, TC ($P<0.001$), WBC ($P<0.05$), Lymphocytes and Monocytes ($P<0.005$), and significant effects of storage time (T0 and T24) on MCH, MCHC, TC, WBC, Lymphocytes, Monocytes and Neutrophils ($P=0.005$). By the results of this study, it was found that haematological parameters and differential leukocyte count of *M. cephalus* were influenced by anticoagulants used and that, according to our previous study,[5] haematologic determinations should be assessed within 24 hours after collection, because long-term storage modifies the results of the analysis. All haematological parameters values obtained in EDTA, heparin and sodium citrate treated samples at T0 fall within values obtained in our previous study on *M. cephalus*[5] and leukocytes differential count of the samples treated with the three anticoagulants were within fish reference range.[6] Thus, haematologic determination and leukocyte differential count showed no cellular alteration depending on used anticoagulants. However studied parameters showed a higher reliability using EDTA as anticoagulant. Comparison of literature data indicates that modifications of blood parameters induced by internal or/and external factors, such as anticoagulants and storage time, could represent a species-specific responses. Further studies designed specifically to investigate the impact of different anticoagulants and storage times on these parameters could be still needed in various fish species to validate an appropriate method for haematological analysis useful for the evaluation of the health status of animal living in captivity and in aquaculture.

References

1. Wood BL, Andrews J, Miller S, Sabbath DE. Refrigerated storage improves the stability of complete blood count and automated differential. Am J Clin Pathol 1999;112:687-95.
2. Korcock DE, Houston AH, Gray JD. Effects of sampling conditions on selected blood variables of rainbow trout, *Salmo gairdneri* Richardson. J Fish Biol 1988;33:319-330.
3. Walencik J, Witeska M. The effects of anticoagulants on hematological indices and blood cell morphology of common carp (*Cyprinus carpio* L.). Comp Biochem Physiol Part C 2007;146:331-5.
4. Ekanem AP, Udoh AJ, Inyang-Etoh AP. Effect of different anticoagulants on hematological parameters of *Oreochromis Niloticus.* Int J Sci Advan Technol 2012;2:17-20.
5. Faggio C, Casella S, Arfuso F, et al. Effect of storage time on haematological parameters in mullet, *Mugil cephalus.* Cell Biochem Funct 2013;31:412-6.
6. Smith C. Hematology and cell type differentiation. In: Mumford S, Heidel J, Smith C, Morrison J, MacConnell B, Blazer V, eds. Fish histology and histopathology. USFWS-NCTC; 2007. pp 1-12.

Algal pigments as dye sources in the solar photovoltaic technology

S. Armeli Minicante,[1] I. Citro,[2] M. Morabito,[3] G. Genovese,[3] G. Calogero[2]

[1]Department of Environmental Sciences, Informatics and Statistics, University Ca' Foscari, Venice; [2]CNR-IPCF Institute for Chemical-Physical Processes, Messina; [3]Department of Biological and Environmental Sciences, University of Messina, Italy

Macroalgae show a broad range of applications and their importance in several sectors is steadily increasing worldwide. Aquaculture produced 15.8 million tonnes of aquatic plants, with a total estimated value of US$ 7.4 billion.[1] About 47% of the total production is used for human consumption, 43% in the extraction of colloids, 7% in the production of maërl and the remaining 3% in the fields of pharmacology, cosmetics, agriculture and waste water treatments from aquaculture, sewage, agricultural and industrial run-off. Algal biomass are also employed in the field of renewable energies for the production of biogas. Among alternative energies, the solar photovoltaic technology is one such source that can looked up to as vast research is being carried out and a significant improvement in performance has been achieved. However, macroalgae are an underexploited resource in the photovoltaic technologies.[2,3]

Dye-sensitized nanocrystalline solar cells (DSSCs) has become an important topic in solar cell research. DSSCs was inspired by the energy and electron transfer mechanisms in natural photosynthesis, and their are based on the photosensitization of nanocrystalline TiO2 semiconductor electrodes by dyes.[4] In nature, some fruits, flowers and leaves contain several pigments that can be easily extracted and employed in DSSCs. Many reports have showed that chlorophyll, which acts as an effective photosensitizer in photosynthesis, has the potential to be an environment friendly dye sources.[5-7] Presently, macroalgae are an underexploited resource in comparison to crop plants.

In the present study, we utilized chlorophylls from samples of the brown alga *Undaria pinnatifida* as sensitizer in DSSCs to investigate the light to electron conversion efficiency. Samples of *U. pinnatifida* were collected in Venice Lagoon (45°26' N; 12°20' E); chlorophylls were extracted by treatment with acetone, according to the protocol of Wang *et al.*[3] The dye,

extracted by frozen seaweeds and used without any chemical purification, showed a very good fill factor (0.69). Even the photelectrochemical parameters if compared with the existent literature are very interesting.

U. pinnatifida is a highly invasive species and has caused concern all over the world because it has invaded coastal environments, has the potential to displace native species, significantly alters habitat for associated fauna, and disturbs navigation. An exploitation of *U. pinnatifida* would result in the conversion of a waste into a valuable biomass.

References

1. FAO. The state of world fisheries and aquaculture 2012. FAO: Rome; 2012.
2. Kuo C-G, Sheen B-J. Seaweed chlorophyll on the light-electron efficiency of DSSC. Journal of the Chinese Chemical Society 2011;58:186-90.
3. Wang X-F, Zhan C-H, Maoka T, et al. Fabrication of dye-sensitized solar cells using chlorophylls *c1* and *c2* and their oxidized forms *c'1* and *c'2* from *Undaria pinnatifida* (Wakame). Chemical Physics Letters 2007;447:79-85.
4. Grätzel M. Dye-sensitized solar cells. Journal of Photochemistry and Photobiology C: Photochemistry Reviews 2003;4:145-53.
5. Calogero G, Di Marco G, Caramori S, et al. Natural dye senstizers for photoelectrochemical cells. Energy & Environmental Science 2009;2:1162-72.
6. Calogero G, Di Marco G, Cazzanti S, et al. Efficient dye-sensitized solar cells using red turnip and purple wild Sicilian prickly pear fruits. International Journal of Molecular Sciences 2010;11:254-67.
7. Narayan MR. Review: dye sensitized solar cells based on natural photosensitizers. Renewable and Sustainable Energy Reviews 2012;16:208-15.

Correspondence: Simona Armeli Minicante, Department of Environmental Sciences, Informatics and Statistics, University Ca' Foscari, Dorsoduro 2137, 30123 Venice, Italy.
E-mail: simona.armeli@unive.it

Daily rhythm of total activity/rest pattern in small and large domestic animals

M. Bazzano,[1] C. Faggio,[2] M. Panzera,[1] A. Assenza,[1] G. Piccione[1]

[1]Department of Veterinary Sciences, University of Messina; [2]Department of Biological and Environmental Sciences, University of Messina, Italy

Circadian rhythms represent an inherent property of living organisms that seem to guarantee an optimal functioning of the biological system, with maximum efficiency, performance and welfare.[1] In mammals, a master clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus adjusts the timing of other self-sustained oscillators in the brain and peripheral organs.[2] In most species, the daily light-dark (L/D) cycle is the primary environmental stimulus (Zeitgeber) for the entrainment of the SCN pacemaker. The SCN receives light information from the retina and regulates several physiological processes by synchronizing molecular clockwork mechanisms consisted by a core group of clock genes in each cell.[3] Among all physiological processes, the total locomotor activity (TLA) is one of the most susceptible to the L/D cycle. Light acutely suppresses locomotor activity in nocturnal (night active) animals such as rats and owls but promotes activity in diurnal (day active) animals like dogs and eagles.[4] Since animals have a species-typical organization of activity patterns,[5] the aim of this study was to compare the TLA in small and large domestic animals like rabbits, cats, dogs, goats, sheep, cows, donkeys and horses. Five clinically healthy female subjects from eight different species: rabbits (body weight 2.5±0.2 kg), cats (body weight 4.5±0.3 kg), dogs (body weight 13.5±1 kg), goats (body weight 40±2 kg), sheep (body weight 45±2 kg), cows (body weight 390±10 kg), donkeys (body weight 395±20) and horses (body weight 565±42) were enrolled in the study with owners consent. Animals were housed under natural photoperiod (March) 12:12 hours L/D cycle (5.30 am sunrise, 5.30 pm sunset) according to specific farm management, except for cats and dogs that lived outdoors. Water was available *ad libitum* and feeding was suitable for each species. Total activity pattern was recorded for 10 days using actigraphy-based data loggers Actiwatch-Mini (Cambridge Neurotechnology Ltd, UK) placed on each animal through collars or halters according to the species. Activity was monitored with a sampling interval of 5 minutes. Total daily amount of activity, mount of activity during the photophase and the scotophase were calculated using Actiwatch Activity Analysis 5.06 (Cambridge Neurotechnology Ltd, UK). The Cosine peak of a rhythm (the time of the daily peak) was computed by cosinor rhythmometry[6] as implement in the Actiwatch Activity Analysis 5.06 program. The temporal resolution of the locomotor activity data was reduced to t h bins by the averaging of all 15 data points within each 1 h bin to apply the statistical analysis. To analyze the locomotor activity a trigonometric statistical model was applied to each time series to statistically describe the periodic phenomenon, by characterizing the main rhythmic parameters according to the single cosinor procedure.[6] Four rhythmic parameters were determined: mean level, amplitude, acrophase (the time at which the peak of a rhythm occurs), and robustness (strength of rhythmicity). For each animal, the mean level of the rhythm was computed as the arithmetic mean of all values in the data set (24 data points). The amplitude of the rhythm was calculated as half the maximum-minimum range of the oscillation, which was computed as the difference between peak and trough. Robustness was computed as the percentage of the maximal score attained by the chi-square periodogram statistic for ideal data sets of comparable size and 24-h periodicity.[7] Two-way analysis of variance (ANOVA) was used for the assessment of effects due to species and days on the daily amount of activity per 24 h. Statistical analysis showed significant differences among domestic species. The highest daily amount of activity was observed during the photophase ($p<0.0001$) in dogs, sheep, goats, cows, donkeys and horses, and during the scotophase ($p<0.0001$) in rabbits and cats. Our results show different pattern of locomotor activity in every domestic species (Figure 1), underlining a diurnal pattern of locomotor activity in dogs, goats, sheep, cows, donkeys and horses while rabbits and cats have a main nocturnal pattern. As previously observed by several authors,[8-10] our study confirms that locomotor activity exhibits a robust daily rhyth-

Correspondence: Giuseppe Piccione, Department of Veterinary Sciences, University of Messina, Polo Universitario dell'Annunziata, Viale Annunziata, 98168 Messina, Italy.
E-mail: gpiccione@unime.it

Journal of Biological Research 2015; 88:5161

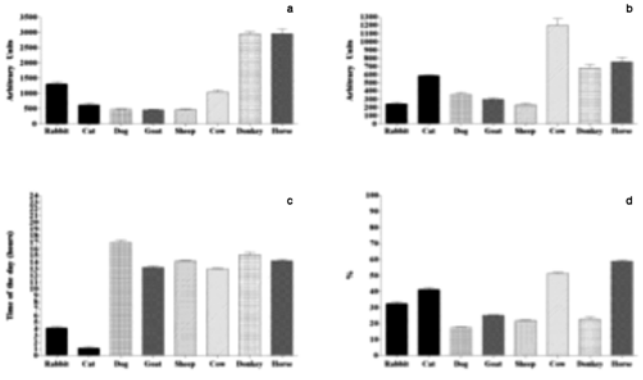

Figure 1. Analysis of rhythm parameters in domestic species: a. Total Locomotor Activity, b. Amplitude, c. Acrophase, d. Robustness.

micity during the photophase in dogs, cows and horses, therefore in these species the rhythm can be poorly affected by external stimuli. On the contrary, other domestic species can spontaneously shift from diurnal to nocturnal activity pattern. Sheep with restricted night time feeding can shift the main bout of activity during the night[8] or cats, that are considered mainly nocturnal, use to loose their rhythm when they live in symbiosis with humans.[11] Therefore, the daily pattern of TLA does not depend only on L/D cycle but it can be affected by several environmental variables including different activities such as feeding, drinking, walking, grooming, playing as well as all conscious and unconscious movements.

References

1. Weinert D, Waterhouse J. The circadian rhythm of core temperature: Effects of physical activity and aging. Physiol Behav 2007;90:246-56.
2. Challet E. Interactions between light, mealtime and calorie restriction to control daily timing in mammals. J Comp Physiol B 2010;180:631-44.
3. Murphy BA, Martin AM, Elliot JA. Light: dark, circadian, and ultradian regulation of motor activity and skeletal muscle gene expression in the horse. J Equine Vet Sci 2009;29:313-4.
4. Refinetti R. The diversity of temporal niches in mammals. Biol Rhythms Res 2008;39,173-92.
5. Giannetto C, Casella S, Caola G, Piccione G. Photic and non-photic entrainment on daily rhythm of locomotor activity in goats. Anim Sci J 2010;8:122-8.
6. Nelson W, Tong U, Lee J, Halberg F. Methods for cosinor rhythmometry. Chronobiol 1979;6:305-23.
7. Refinetti R. Non-stationary time series and the robustness of circadian rhythms. J Theor Biol. 2004; 227, 571-581.
8. Piccione G, Giannetto C, Casella S, Caola G. Daily locomotor activity in five domestic animals. Anim Biol 2010;60:15-24.
9. Siwak CT, Tapp PD, Zicker SC, et al. Locomotor activity rhythms in dogs vary with age and cognitive status. Behav Neurosci. 2003;117:813-24.
10. Zanghi MB, Kerr W, de Rivera C, et al. Effect of age and feeding schedule on diurnal rest/activity rhythms in dogs. J Vet Behav 2013;8:195-203.
11. Piccione G, Marafioti S, Giannetto C, et al. Daily rhythm of total activity pattern in domestic cats (Felis silvestris catus) maintained in two different housing conditions. J Vet Behav 2013;8:189-94.

Selection of the best oocytes for intracytoplasmicsperm injection (ICSI) using apoptotic analysis of cumulus cells

L. Bosco,[1] G. Ruvolo,[2] R. Chiarelli,[1] M. Agnello,[1] M.C. Roccheri[1]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [2]Center of Reproductive Biology, Palermo, Italy

Introduction

We studied the apoptosis rate of the cumulus cells of individual cumulus-oocyte complex (COC), to verify a relationship with clinical outcomes, in terms of pregnancy and implantation rates. Usually oocytes are selected using morphological criteria. We tried to verify if cumulus cell apoptotic rate could be used as molecular criteria in selecting oocytes with higher implantation potentiality.[1,2]

Materials and Methods

The study design consisted in two different trials: in the first, we investigated apoptosis rate in cumulus cells of the three selected oocytes, to be fertilized by intracytoplasmic sperm injection (ICSI); in a second trial, average apoptosis rate of the cumulus cells coming from the three selected oocytes to be fertilized by ICSI and the pooled remaining oocytes were compared, when more than 5 COCs were aspirated. In a first trial we included 22 consecutive couples undergoing ICSI cycles, 20 in a second one, for a total of 42 patients. We selected the three oocytes for (ICSI) on the basis of the morphological appearance of the cumulus, according to Veek's criteria. The cumulus cells of each COC were submitted to apoptotic assays.[3] The patients were classified, on the basis of pregnancy success, in A Group (pregnant patients) and B Group (patients with negative hCG).

Results and Discussion

Both trials showed that apoptosis in the cumulus cells was remarkably lower in the A Group if compared with B Group. The apoptosis rate in the selected COCs was similar to pooled COCs for each patient, confirming that apoptosis rate in cumulus cells is characteristic for patient. Out of 22 patients involved in the first trial, 8 were pregnant (36.3% A Group) and 14 were not pregnant (B Group). In the second trial 4 of a total of 20 patients were pregnant (20%). In the first trial a total of 58 metaphase II oocytes and 56 in the second trial were studied. In the second trial 38 oocytes where pooled to compare apoptosis rate with the three selected oocytes pools. In the first trial the incidence of DNA fragmentation, evaluated by TUNEL assay (Figure 1), of the cumulus cells from individual treated oocytes, was lower in A Group than in B Group (6.7% ranging between 2.2-13.3 *vs* 13.19% ranging between 6.2-34.9 respectively, $p<0.05$). To confirm if DNA fragmentation was related to apoptosis process, we performed caspase-3 immunoassay in the same cells (Figure 2). Data showed a lower capase-3 activity in cumulus cells of pregnant than in those of non-pregnant patients (5.2% ranging between 1.2-8.6 *vs* 11.8% ranging between 5.6-14.8, $p<0.05$). It is noteworthy to underline that pregnant patients usually exhibited, at least, one COC with a DNA fragmenta-

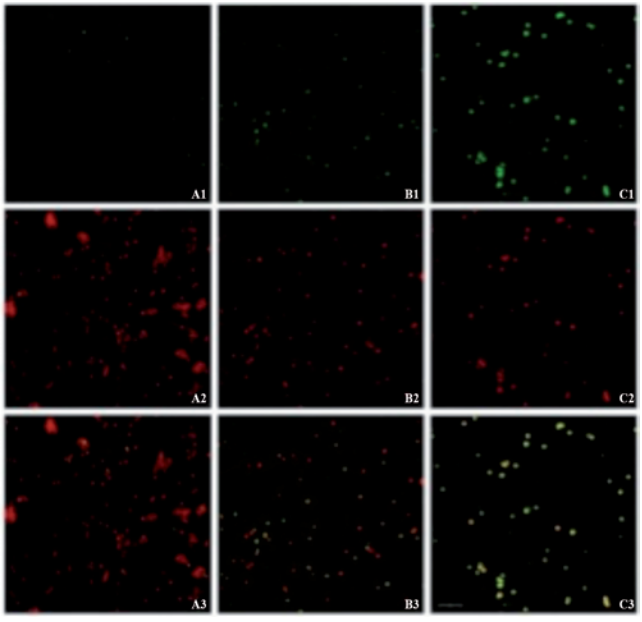

Figure 1. Apoptosis evaluation using TUNEL assay in human cumulus cells. (A1, A2, A3) A group; (B1, B2, B3) B group; (C1, C2, C3) positive control for TUNEL assay. (A1, B1, C1) fragmented DNA; (A2, B2, C3) propidium iodide staining; (A3, B3, C3) merge. Scale bar=15 µm.

Correspondence: Liana Bosco, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Viale delle Scienze 16, 90128 Palermo, Italy.
E-mail: jurkart@hotmail.com

Journal of Biological Research 2015; 88:5161

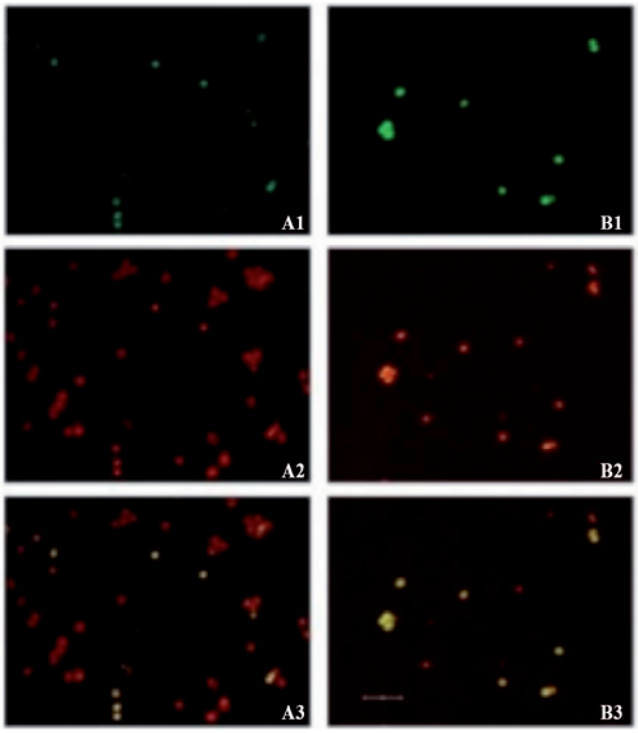

Figure 2. Apoptosis evaluation using Cleaved caspase 3 immunofluorescence *in situ* assay in human cumulus cells. (A1, A2, A3) A group; (B1, B2, B3) B group; (A1, B1) Cleaved caspase 3; (A2, B2) propidium iodide staining; (A3, B3) merge. Scale bar=15 µ.

tion rate (TUNEL) less than 10% and caspase-3 activity rate less than 7%. Four (A Group) of 20 patients involved in the second trial were pregnant but two aborted at 8-9 weeks. The low number of pregnant patients did not allow us to have a powerful statistical analysis of apoptotic rate in cumulus cells, but it seems evident that a higher apoptotic rate in cumulus cells is associated to the pregnancy failure (B Group) and in aborted patients of A Group, ranging from 10 to 60.3%.

Conclusions

The data seem to demonstrate that apoptosis may be a marker for the selection of the best oocytes to be submitted to ICSI treatment. All pregnant patients showed a lower apoptosis rate in cumulus cells if compared with patients with pregnancy failure.

References

1. Ruvolo G, Bosco L, Pane A, et al. Lower apoptosis rate in human cumulus cells after administration of recombinant luteinizing hormone to women undergoing ovarian stimulation for in vitro fertilization procedures. Fertil Steril 2007;87:542-6.
2. Host E, Gabrielsen A, Lindenberg S, Smidt-Jensen S. Apoptosis in human cumulus cells in relation to zona pellucida thickness variation, maturation stage, and cleavage of the corresponding oocyte after intracytoplasmic sperm injection. Fertility and Sterility 2002;77:511-5.
3. Bosco L, Ruvolo G, Morici G, et al. Apoptosis in human unfertilized oocytes after intracytoplasmic sperm injection. Fertil Steril 2005;84:1417-23.

Application of multivariate T-pattern analysis in the study of social interaction in rats

M. Casarrubea,[1,2] D. Cancemi,[3] A. Cudia,[3] M. Cardaci,[2,3] F. Sorbera,[1] F. Faulisi,[1] M.S. Magnusson,[4] G. Crescimanno[1,2]

[1]Human Physiology Unit, Department of Experimental Biology and Chemical Neurosciences, University of Palermo; [2]Department of Psychology, University of Palermo, Italy; [3]Human Behavior Laboratory, University of Iceland, Reykjavik, Iceland; [4]Interdepartmental Center of Technologies of Knowledge, University of Palermo, Italy

Introduction

The social interaction test, introduced by File and Hyde in 1978,[1] is a well known behavioral model used to investigate anxiety-related behaviors in rodents. Basic premise of the test is that the behavior of a rodent influences the one of the other.

An increased interaction between the two subjects is indicative of a reduced anxiety condition, in contrast, a reduced interaction indicates a higher anxiety. Albeit the test has been largely used to study anxiolytic and neuroleptic drugs, no data are available on the temporal structure of the behavioral interactions. In addition, interesting questions still remain unanswered.

For instance, is it possible to identify recurring temporal sequences from the two interacting rodents? If so, do such sequences encompass specific behavioral events? Could such sequences have an ethological meaning?

Materials and Methods

In this preliminary study, four male Wistar rats (220-250 gr) were used. Each subject was housed in a thermo-regulated room. The day of the experiment, pairs of rats, randomly taken from housing room, were placed in an open field apparatus (50 x 50 cm) and observed for 15 min. The behavior of the animals was recorded with a digital video camera and video files stored in a personal computer. Using an ethogram, obtained on the basis of previous observations,[2-5] video files were coded by means of a software coder and the obtained event log files used for following analyses. Both quantitative and multivariate t-pattern analyses were carried out. The latter is a multivariate approach based on the utilization of a specific software that, by means of an advanced search algorithm, processes event log files evaluating possible significant relationships among the events in the course of time.[6] Theories, concepts and procedures concerning such a multivariate analysis of behavior can be found in our previous articles.[6-8]

Correspondence: Giuseppe Crescimanno, Human Physiology Unit, Department of Experimental Biology and Chemical Neurosciences, University of Palermo, Via del Vespro 129, 90127 Palermo, Italy.
Tel: +39.091.6555847.
E-mail: giuseppe.crescimanno@unipa.it

Journal of Biological Research 2015; 88:5161

Results

The ethogram is presented in Table 1. The behavioral activities are classified taking into consideration their characteristics: *non social* (produced in absence of interactions), and *social* (produced during interactions).

Preliminary results, obtained from the analysis of two pairs of rats, are presented. Per cent distribution, evaluated both for social and non social activities are illustrated in Figure 1. ISn, Wa, Cl, Re, FPL, Imm and Sh represent 95.50% of the non social behavioral repertoire; on the other hand, SoS, App, Wit, LeO, SoG and GeS represent 84.50% of the social one. T-pattern analysis demonstrated, in both pairs of rats, the presence of significant constraints among numerous events in the course of time. Figure 2 illustrates a t-pattern detected in one pair of rats.

Discussion

The current research represents the first effort to study the temporal structure of social interaction in rats by means of multivariate t-pattern analysis. Per cent distribution (Figure 1) shows that sniffing related (ISn, Cl, Re, SoS, GeS) and walking related (Wa, App, Wit) activities (Table 1) are the most represented both in non social and social behavior.

Coherently, the t-pattern presented in Figure 2, encompassing only sniffing- and walking-related activities, well depicts the role of these behavioral events in moulding the temporal structure of the behavior. Interestingly, some kinds of behavioral symmetries were observed, where a behavior, carried out by one of the rats, was followed by the same behavior carried out by the other animal (Figure 2).

Table 1. Ethogram. First column: behavioral element. Second column: abbreviation. Third column: description.

Non Social	Abbr.	Description
Walking	Wa	The rat walks around sniffing the environment
Climbing	Cl	The rat maintains an erect posture leaning against the Plexiglas wall. Usually associated with sniffing.
Rearing	Re	The rat maintains an erect posture without leaning against the wall. Usually associated with sniffing.
Front Paw Licking	FPL	The rat licks or grooms its forepaws
Hind Paw Licking	HPL	The rat licks or grooms its hind paws
Face Grooming	FGr	The rat ribs its face with the forepaws
Body Grooming	BGr	The rat rubs the body combing the fur by fast movement of the incisors
Shaking	Sh	The rat shakes its head and body with rapid semicircular movements
Immobility/Resting	Imm	The rat maintains a fixed posture
Immobile Sniffing	ISn	The rat sniffs the environment, standing on the ground
Social	**Abbr.**	**Description**
Withdrawing	Wit	One rat walks or runs away from the other rat.
Following	Fol	One rat follows the partner while the other is walking away
Approaching	App	One rat walks in the direction of the partner, while the other rat is immobile or is alredy approaching him
Crawling over	CrO	One rat walks over the partner
Crawling under	CrU	One rat walks under the partner
Boxing/Wrestling	Box	Offensive/aggressive behaviors such as pawing, pouncing, nosing, biting, boxing, kicking, wrestling
Leaning on	LeO	One of the rats leans with its forelimbs on the other rat that, in turn, maintains all the four paws on the ground.
On-top	Top	One of the animals stands over the partner that lies with its back on the floor
On-back	Bck	One of the animals lies with its back on the floor with the other animal standing over it
Mounting	Mnt	One of the rats holds the other rat's trunk with the forelimbs
Social grooming	SoG	One of the rats grooms its partner's body, neck or face
Social sniffing	SoS	One of the rats sniffs the partner's face and/or body
Ano-genital sniffing	GeS	One of the rats sniffs the partner's anogenital area

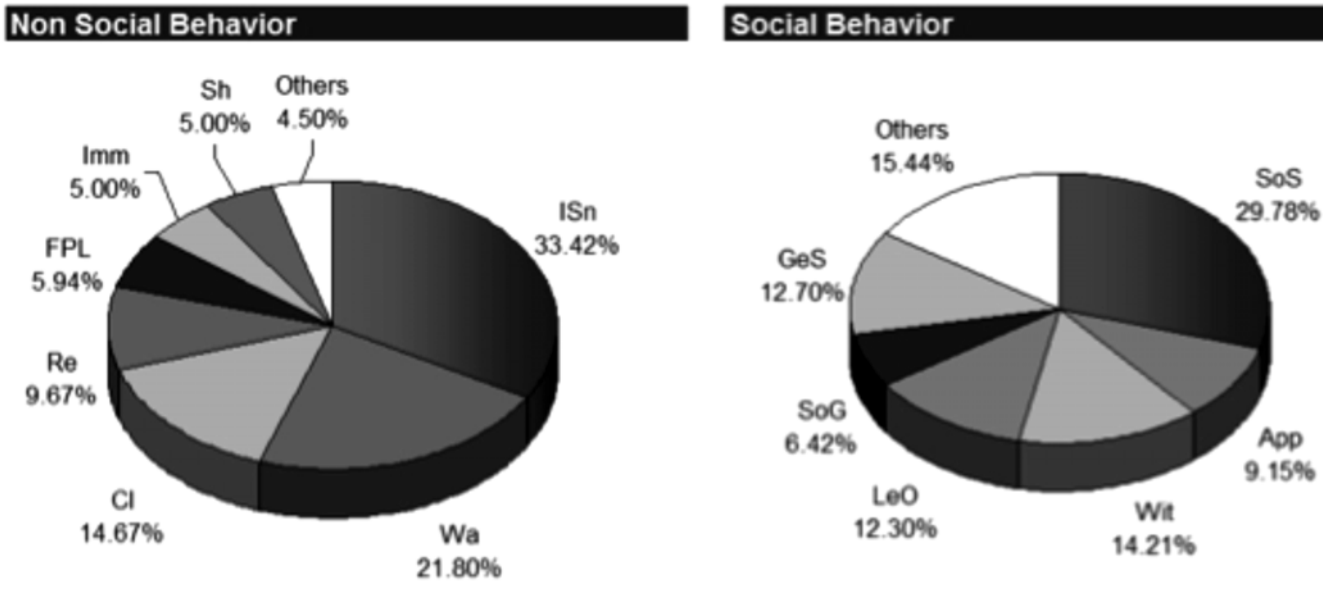

Figure 1. Per cent distribution of non social (left pie) and social (right pie) behavioral elements carried out by the rats. Others=behavioral elements <5%. For abbreviations see Table 1.

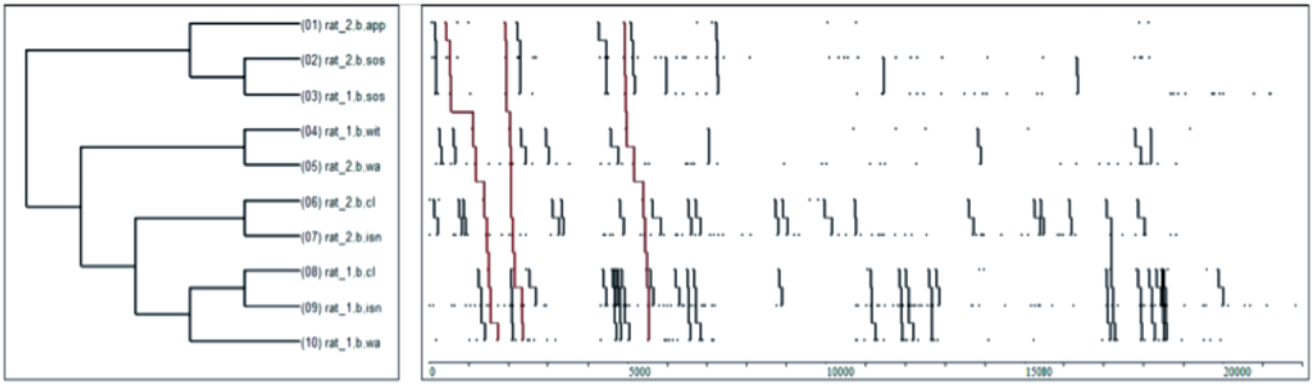

Figure 2. T-pattern detected in one of the two pairs of rats. Left box: tree structure. Number in brackets indicates the order of appearance of each event. Right box: connection diagram. Dots show the occurrences of the corresponding events reported in the left box. For abbreviations see Table 1.

Conclusions

To sum up, preliminary results obtained from t-pattern analysis demonstrate that the behavior of rats in the social interaction test is extremely complex and structured on the basis of numerous, recurring and statistically significant sequences of events.

References

1. File SE, Hyde JR. Can social interaction be used to measure anxiety ? Br J Pharmacol 1978;62:19-24.
2. Spruijt BM. Progressive decline in social attention in aging rats: an information-statistical method. Neurobiol Aging 1992;13:145-51.
3. Vanderschuren LJ, Spruijt BM, Hol T, et al. Sequential analysis of social play behavior in juvenile rats: effects of morphine. Behav Brain Res 1995;72:89-95.
4. Van Den Berg CL, Van Ree JM, Spruijt BM. Sequential analysis of juvenile isolation- induced decreased social behavior in the adult rat. Physiol Behav 1999;67:483-8.
5. Yamada-Haga Y. Characteristics of social interaction between unfamiliar male rats (Rattus norvegicus): comparison of juvenile and adult stages. J Ethol 2002;20:55-62.
6. Magnusson MS. Discovering hidden time patterns in behavior: t-patterns and their detection. Behav Res Methods Instrum Comput 2000;32:93-110.
7. Casarrubea M, Roy V, Sorbera F, et al. Temporal structure of the rat's behavior in elevated plus maze test. Behav Brain Res 2013;237:290-9.
8. Casarrubea M, Roy V, Sorbera F, et al. Significant divergences between the temporal structure of the behavior in Wistar and in the spontaneously more anxious DA/Han strain of rats tested in elevated plus maze. Behav Brain Res 2013;250:166-73.

IL-1β induces DNA demethylation, at genome level and in specific CpG sites of IL-6 and IL-8 genes in human intestinal epithelial cells

I. Schifano, C. Gentile, F. Naselli, L. Sposito, M. Allegra, L. Tesoriere, M.A. Livrea, F. Caradonna

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

Inflammation is a complex physiological response that requires the activity of a sophisticated regulatory network involving the activation of specific genes for defense, tissue repair and remodeling.

Although transcriptional activation has been shown to be critical in the regulation of inflammatory genes[1] the role of epigenetic phenomena in the modulation of the inflammatory response is now emerging.[2] Specifically, it has been recently reported that proinflammatory stimuli induce DNA demethylation in the interleukin IL-1β promoter of human articular chondrocytes.[3] IL-1β cytokine, among several proinflammatory agents, represents an essential player in the inflammatory conditions of the gut:[4] functioning as the strongest signal transduction to NF-kB, IL-1β increases in intestinal paracellular permeability and over- expression of proinflammatory genes.[5] In this tissue, moreover, inflammatory response is crucial to maintain its structural integrity and function, thus, alteration and deregulation of inflammatory pathways contribute to tissue damage and ulceration, and are pivotal factors in the pathogenesis of several inflammatory gut diseases.

In the present study we evaluate both wide-ranging and gene-specific epigenetic changes in the inflammatory response of Caco-2 cells differentiated into intestinal epithelial cells and exposed

to the inflammatory actions of IL-1β. Our results clearly show that IL-1β induces changes in the DNA methylation either at genome and gene level and that the local methylation changes are induced in two pro-inflammatory genes that are IL-1β-regulated. In particular, we show that a cell exposure to IL-1β for 24 h induces, in a dose-dependent manner, hypomethylation of genomic DNA in respect to untreated cells. We also observe a reduced DNA methyltransferases activity of cell lysates obtained from IL-1β that IL-1β treated cells . Finally our data shows that IL-1β is able to induce hypomethylation of specific CpG sites in IL-6 and IL-8 genes.

These preliminary results suggest that IL-1β in intestinal epithelial cells is able to act as an epigenetic modulator towards the entire genome and specific genes.

Modulation of epigenetic changes in inflammation may provide a new *reading frame* of the inflammatory diseases molecular basis. Since epigenetic modifications are potentially reversible, a thorough understanding of these changes during inflammatory response opens opportunities to develop efficient agents for specific targets.

References

1. Medzhitov R, Horng T. Transcriptional control of the inflammatory response. Nat Rev Immunol 2009;9:692-703.
2. Bayarsaihan D. Epigenetic mechanisms in inflammation. J Dent Res 2011;90:9-17.
3. Hashimoto K, Oreffo RO, Gibson MB, et al. DNA demethylation at specific CpG sites in the IL1B promoter in response to inflammatory cytokines in human articular chondrocytes. Arthritis Rheum 2009;60:3303-13.
4. Ligumsky M, Simon PL, Karmeli F, Rachmilewitz D. Role of interleukin 1 in inflammatory bowel disease-enhanced production during active disease. Gut 1990;31:686-9.
5. Van De Walle J, Hendrickx A, Romier B, et al. Inflammatory parameters in Caco-2 cells: effect of stimuli nature, concentration, combination and cell differentiation. Toxicol In Vitro 2010;24:1441-9.

Correspondence: Fabio Caradonna, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Viale delle Scienze 16, 90128 Palermo, Italy.
E-mail: fabio.caradonna@unipa.it

Journal of Biological Research 2015; 88:5161

Design and synthesis of fluorescent GPER ligands as useful tools in molecular biology and drug discovery process

A. Barattucci,[1] P. Bonaccorsi,[1] S. Campagna,[1] D. Carbone,[1] T. Papalia,[2] F. Puntoriero,[1] C. Rosano[3]

[1]Department of Chemical Sciences, University of Messina; [2]Department of Pharmaceutical and Health Products Sciences, University of Messina; [3]National Institute for Cancer Research, Genoa, Italy

Breast and ovarian tumors are among the major cause of death in Western Countries' women. Estrogens play a pivotal role in the development of these hormone-sensitive tumors. Recently, a seven-transmembrane G-protein-coupled receptor (GPCR), named GPER, has been identified as membrane estrogen receptor able to mediate rapid estrogen signalling in a variety of normal and cancer cell types.[1] Different studies showed that GPER promotes the up-regulation of the oncogene *c-fos* and stimulates proliferative effects induced by estrogens and antiestrogen in cancer cells such as breast, endometrial and thyroid.[2] GPER is a 7 helices transmembrane protein (7TM) and its cellular localization is still a matter of debate: although it has been found within the Endoplasmic Reticulum membrane, further studies demonstrated the presence of this receptor on the cellular membrane. GPER has been associated with the proliferative effects induced by 17β-estradiol (E2) and a selective ligand of GPER, G-1, (Figure 1A) through a functional cross-talk with ERα in ovarian cancer cells, playing an important role in tamoxifen- resistant breast cancer cells.[3]

Taken together, these data indicate that GPER plays a role in a complex transduction network which mediates the biological responses to estrogens. However, the mechanism of activation of signals, the structure-function relationships, the pharmacological spectrum regarding GPER still suggest questions to be solved. The possibility to develop small molecular probes, to facilitate elucidation of mechanistic pathways and enable specific manipulation of the activity of GPER, provides an extraordinary tool in the complex field of drug discovery. Biological information can offer a more detailed scenery if the classical and efficient method of investigation, based on the exposition of cells to a fluorescent dye, such as one of the commonly used Alexa family,[3] can be flanked to the use of luminescent ligands of the protein under study.

In this communication we describe the synthesis of a family of small GPER ligands with intrinsic fluorescent properties. This approach can be regarded as a useful tool for exploiting structural changes of a protein upon interaction with specific target molecules and developing new targeted imaging agents for the intracellular receptor. We started in designing small molecules (Figure 2) showing the structural characteristics of some already known GPER ligands, such as the agonist G1 and the antagonist G15 (Figure 1B), but with fluorescent elements incorporated in their skeleton, trying to avoid alteration in their properties as GPER agonists or, better, antagonists. For the fluorescence, we have been inspired by the dipyrromethene boron difluoride (BODIPY) dyes (Figure 1C) as strongly UV-visible absorbing small fluorophores that exhibit relatively sharp fluorescence with high quantum yield,[4] are reasonably stable under physiological conditions and have been widely investigated as fluorescent probes for biological studies.

In Figure 3 the two synthetic pathways a) and b), for the preparation of compounds 1, are shown. This communication will describe the details of these syntheses, the results gained and the work that has to be ended. The design of the fluorescent GPER ligands has been supported by virtual screening of their potentially effective molecular skeletons.

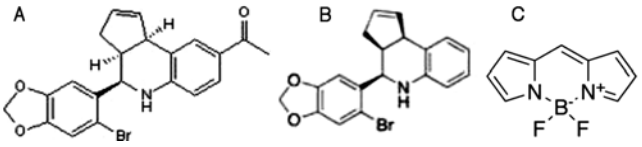

Figure 1. A: GPER agonist G-; B: GPER antagonist G-15; C: the skeleton of fluorescent BODIPY.

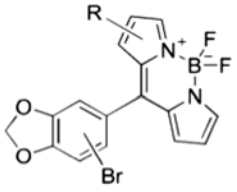

Figure 2. The general skeleton of the projected GPER fluorescent ligands.

Correspondence: Donatella Carbone, Department of Chemical Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres 31, 98166 Messina, Italy.
E-mail: donatellacarbone@libero.it

Journal of Biological Research 2015; 88:5161

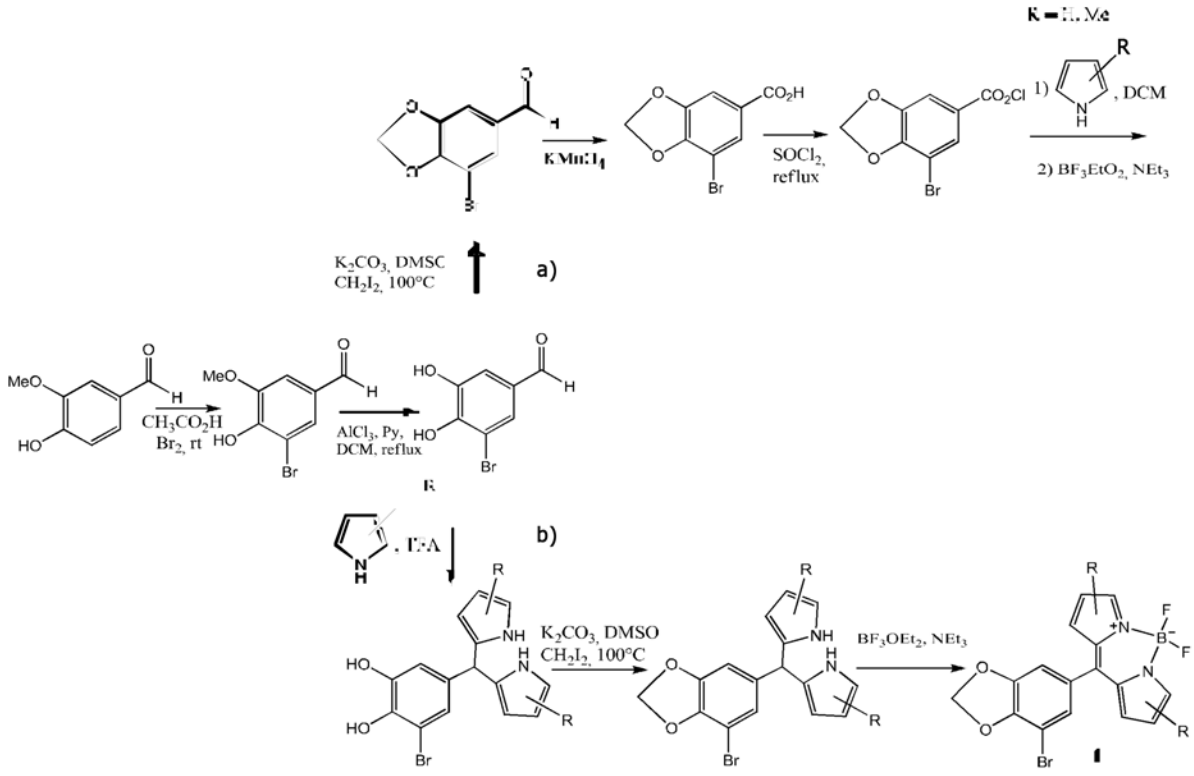

Figure 3. Synthetic pathways to fluorescent GPER ligands.

References

1. Wang D, Hu L, Zhang G, et al. G protein-coupled receptor 30 in tumor development. Endocrinology 2010;38:29-37.
2. Maggiolini M, Picard D. The unfolding of GPR30, a new membrane-bound estrogen receptor J Endocrinol 2010;204:105-14.
3. Filardo E, Quinn J, Pang Y, et al. Thomas. Activation of the novel estrogen receptor G protein-coupled receptor 30 (GPR30) at the plasma membrane. Endocrinology 2007;148:3236-45.
4. Bonaccorsi P, Aversa MC, Barattucci A, et al. Artificial light-harvesting antenna systems grafted on a carbohydrate. Platform Chem Commun 2012;48:10550-2.

Forensic technique to alleged human embryo: the first case report in Italy

E. Carra

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

As commissioned by Judicial Authority, it was done an inventory of human cryo-frozen biological material, DNA typing of alleged human embryo and verification of compliance of the findings of PHRs acquired to Prosecution to reconstruct the path followed by gametes and the care of patients who have received medically-assisted reproduction treatments in four Centres highlighting any significant irregularities in contravention of the current legal framework on Assisted Reproduction Technology (ART). This article describes the first forensic investigation in Italy who involves forensic science in Reproductive Medicine,[1,2] taking into account the implementation of the Law 40 of 2004 on ART that has not changed with the pronouncement of The Constitutional Court in May 2009.[3-5] In fact, embryos can be created only for the purpose of procreation and the prohibitions on their cryopreservation, suppression and selection for eugenetic purposes remain.

The inventory of liquid nitrogen containers of human material (semen, oocyte and embryo) has been done in cooperation with police who was video-recording the entire activity. This was done for two reasons: first, in order to be able to demonstrate that the manipulations of canisters, globets, visiotubes and straws, had not affected the cryo-frozen process. On the other hand, this would have allowed reviewing the manipulation, to check what has been writing for the identifications of patients who have received medically assisted reproduction treatments. However, the huge amount of documentation required in seizure had to define correctly the number of cryo-frozen biological material (oocyte and embryo) for each couple of patients to verification of compliance of the findings of PHRs acquired to Prosecution. Eight databases in Access format were found with no information of which centres of Assisted Reproductive could belong to. In addition, the customer registrations of clinical data records were identified by local, idiosyncratic, and sometimes redundant and/or ambiguous names (or codes) rather than unique, well-organized codes from standard code systems. This would have required a process of cleaning up of the data and mapping of the variable names and codes in collaboration with specialists in software programming . We were able to identify the databases corresponding to the 4 centers and we performed a second inventory of the cryo-frozen material to verify the previous data. A few discrepancies were highlighted.

At the same time, a genetic analysis was conducted on an alleged human frozen thawed embryo no more suitable to development. At Prosecution was declared that the human embryo at four cells was irreversibly compromised in its development: the aim of the investigation was to verify the genetic compatibility of a married couple who was waiting for frozen thawed embryo transfer (FET) and had done a complaint to the Prosecutor in order the possibility of replacement with heterologous embryos by medical staff. During the planned Embryo-Transfer (ET) the police put in seizure, one of the three frozen thawed embryo declared in advanced lysis state, into a straw. The laboratory activity was conducted in the presence of three specialists in the interest of the eight Suspects: when we analyzed the straw under microscopy we could not recognize any morphological characteristic of embryo but only small particles in a medium (Figure 1). For some suspects such evidence could fit well but for other suspects did not fit well. Before performing the forensic genetic analysis the cell degeneration was investigated using an animal model (the sea urchin embryo) to understand, after stress conditions (freezing, thawing and physiological involution after death), when the morphological identification of embryo was no more possible. The results demonstrate that for the eggs of sea urchin after 8 days it was impossible to recognize them through microscopy analysis, differently from the embryo at 20 days where small cells could be seen, except that the lysis process was being induced by proteolitic enzymes.

The case was taking aspects of particular relevance since it could not be excluded that the material into the straw in seizure was an oocyte not visible at microscopy. In addition, after the inventory it was proved that three straws with frozen-oocyte belonging to the couple of married patients who had done the complaint were missing. Therefore, only in theory it is simple to demonstrate this, since single cell degradation associated to small amount of forensic DNA is the best

Correspondence: Elena Carra, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Viale delle Scienze 16, 90128 Palermo, Italy.
Tel: +39.091.23897404.
E-mail: elena.carra@unipa.it

Journal of Biological Research 2015; 88:5161

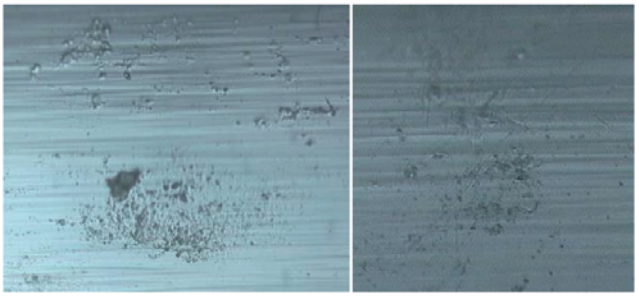

Figure 1. A view of alleged human frozen thawed embryo into seized straw. Magnification 80X - Stereo Microscope LEICA M165FC.

situation to obtain complex DNA profiles. A method of collecting the alleged embryo residues from the straw has been developed conforming to forensic procedure to ensure the repetition of analysis. The sample was collected using Whatman 3MM filter strip paper and was divided in 7 parts under absolute sterile conditions; each strip was extracted using a performed low copy number (LCN) extraction procedure. Low copy number (LCN) typing, particularly for current short tandem repeat (STR) typing, refers to the analysis of any sample that contains less than 200 pg of template DNA. Generally, LCN typing simply can be defined as the analysis of any DNA sample where the results are below the stochastic threshold for reliable interpretation.[6,7] The extracted DNA was amplified using reduced amplification volume and higher PCR. cycle numbers. Autosomal DNA profiles were obtained from most of the 3MM strips. These profiles were concordant with the profiles obtained from the couple of married patients proving the presence of human lysis embryo into the seized straw.

Random analysis on IVF-ET sheets, Clinical Records, IVF Registers, *etc*. combined with the computerization for each type of PHRs of such records: date of pick up, women, man, oocyte, IVF technique, MII oocyte, semen, embryo, discarded oocyte, discarded embryo, frozen-oocyte, frozen-embryo, embryo-transfer ET, MD, Biologist, clinical analysis, note, has permitted to reconstruct the path followed by gametes of patients who had received medically-assisted reproduction treatments highlighting significant irregularities in contravention of the artt. 13 and 14 of the Law 40 of 2004.

References

1. Trounson AO, Wood C. IVF and related technology. The present and the future. Med J Aust 1993;158:853-7.
2. Shufaro Y, Laufer N. Epigenetic concerns in assisted reproduction: update and critical review of the current literature. Fertil Steril 2013;99:605-6.
3. Law 40/2004 - Gazzetta Ufficiale n. 45 del 24 febbraio 2004.
4. Fineschi V, Neri M, Turillazzi E. The new Italian law on assisted reproduction technology (Law 40/2004). J Med Ethics 2005;31:536-9.
5. Robertson JA. Protecting embryos and burdening women: assisted reproduction in Italy. Hum Reprod 2004;19:1693-6.
6. Budowle B, Eisenberg AJ, van Daal A. Validity of low copy number typing and applications to forensic science. Croat Med J 2009; 50:207-17.
7. Gill P. Application of low copy number DNA profiling. Croat Med J 2001;42:229-32.

Effect of γ-aminobutyrric acid exposure on embryogenesis of *Paracentrotus lividus* and identification of γ-aminobutyrric acid-receptor genes in sea urchins

S. Casamirra, G. Spinelli, V. Cavalieri

Department of Biological, Chemical and Pharmacological Science and Technology, University of Palermo, Italy

Developmental processes are controlled by regulatory genes encoding for transcription factors and signaling molecules. Functional relationships between these genes are described by gene regulatory networks (GRN), models which allow integration of various levels of information.[1] The sea urchin embryo is an experimental model system which offers many advantages for the analysis of GRN.[2] Recently, the GRN that governs the biomineralization of the sea urchin embryonic skeleton has begun to be deciphered.[3-5] Preliminary evidence suggest that the γ-aminobutyric acid (GABA) signaling pathway is involved in skeletal morphogenesis during development of the sea urchin. GABA is a molecule synthesized by nearly all organism, from bacteria to humans, and it acts through ionotropic and metabotropic receptors ($GABA_A$-Rs and $GABA_B$-Rs, respectively).[6]

We report that *Paracentrotus lividus* embryos exposed to GABA at concentrations ranging from 0.01 to 1.0 mM showed aberrations in axial patterning, with a dose dependent effect. In particular, at 24 hours post-fertilization (hpf) control embryos displayed two bilateral clusters of Primary Mesenchyme Cells (PMCs; Figure 1Aa), which hold biomineralizing activity. By contrast, treated embryos contained a population of PMCs that was quite homogeneously distributed within the blastocoele (Figure 1Ab). Moreover, at 48 hpf, when control embryos were normal angular-shaped plutei with the characteristic bilateral symmetry (Figure 1Ac), GABA-treated embryos appeared spherical and contained supernumerary spicules (Figure 1Ad).

Washout experiments allowed to determine that the period of sensitivity is restricted from the blastula to the gastrula stage.

In order to identify GABA-R genes we performed a comprehensive *in silico* analysis in selected sea urchin species (*P. lividus, Strongylocentrotus purpuratus,* and *Lytechinus variegatus),* and in phylogenetically related organisms, such as the hemichordate *Saccoglossus kowalevskii*, the chordate *Ciona intestinalis*, and the nematode *Caenorhabditis elegans*.

By combining iteration of *ab initio* predictions and pairwise comparative methods, we identified the orthologous genes encoding for $GABA_{B1}$ and $GABA_{B2}$, the two subunits which assemble $GABA_B$-R, and we confirmed that all of these organisms possess a unique α/β $GABA_A$-R gene pair clustered in the genome. Furthermore, we have observed that the reciprocal disposition of $GABA_A$-R genes is also evolutionarily conserved (Figure 1B).

Interestingly, in adjacent position to these genes, we have identified an additional gene, which shows significant sequence similarity to a invertebrate-specific $GABA_A$-R gene. Indeed, such a gene has been only

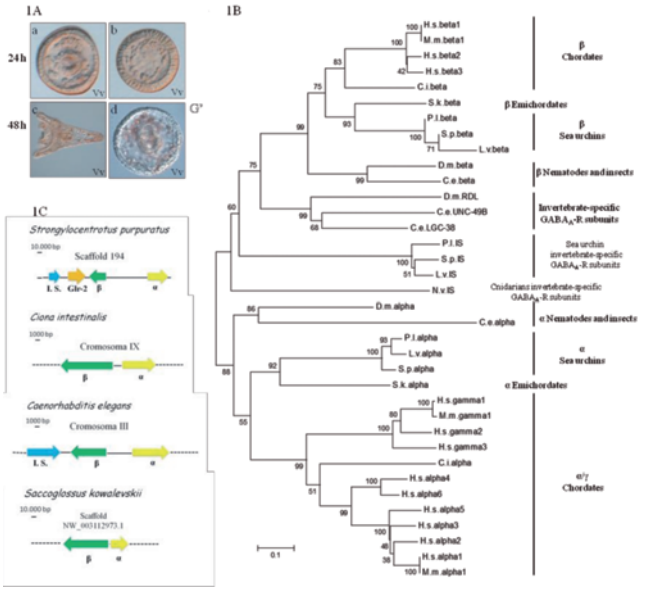

Figure 1. A) Effect of GABA on embryogenesis of *P. lividus*. (a, c), control and (b, d) GABA-treated embryos observed at 24- (a, b) and 48- (c, d) hours post fertilization. Vv: vegetal view. B), Genomic configuration of $GABA_A$-R locus in different species of invertebrates. Glr-2: Glutammate receptor-2; I.S.: invertebrate-specific $GABA_A$-R subunit gene. C) neighbor-joining tree constructed with protein sequences of representative $GABA_A$-R subunits. Number above nodes indicate bootstrap values (1000 replicates). M.m., *Mus musculus*; H.s., *Homo sapiens*; P.l., *Paracentrotus lividus*; S.p., *Strongylocentrotus purpuratus*; L.v., *Lytechinus variegatus*; C.i., *Ciona intestinalis*; S.k, *Saccoglossus kowalevskii*; D.m, *Drosophila melanogaster*; C.e., *Caenorhabditis elegans*; N.v., *Nematostella vectensis*.

Correspondence: Vincenzo Cavalieri, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Viale delle Scienze Ed. 16, Palermo, Italy.
E-mail: vincenzo.cavalieri@unipa.it

Journal of Biological Research 2015; 88:5161

identified in *C. elegans*, *Drosophila melanogaster*, and *Nematostella vectensis*.[7,8]

We also retrieved several cDNA sequences from staged EST databases of the three sea urchin species inspected, indicating that these genes are actively transcribed during development. Some selected cDNA plasmids were also isolated from *P. lividus* total RNA samples and fully sequenced.

Hypothetical proteins were deduced and used for phylogenetic analysis, including a selection of vertebrate and invertebrate GABAA-R subunit sequences. The resulting phylogenetic tree (Figure 1C) strongly support the hypothesis that the sea urchins contain genes encoding for both canonical and invertebrate-specific $GABA_A$-R subunits. Such a collection of data should provide a support to better understand the involvement of GABA-signalling pathway in the skeletal GRN.

References

1. Levine M, Davidson EH. Gene regulatory networks for development. Annu Rev Biophys Biomol Struct 2005;102:4936-42.
2. McClay DR. Evolutionary crossroads in developmental biology: sea urchins. Development 2011;138:2639-48.
3. Duloquin L, Lhomond G, Gache C. Localized VEGF signaling from ectoderm to mesenchyme cells controls morphogenesis of the sea urchin embryo skeleton. Development 2007;134:2293-302.
4. Röttinger E, Saudemont A, Duboc V, et al. FGF signals guide migration of mesenchymal cells, control skeletal morphogenesis and regulate gastrulation during sea urchin development. Development 2008;135:353-36.
5. Cavalieri V, Guarcello R, Spinelli G (2011). Specific expression of a TRIM-containing factor in ectoderm cells affects the skeletal morphogenetic program of the sea urchin embryo. Development 138:4279-90.
6. Watanabe M, Maemura K, Kanbara K, et al. GABA and GABA receptors in the central nervous system and other organs. Int Rev Cytol 2002;213:1-47.
7. Tsang SY, Ng SK, Xu Z, Xue H. The evolution of GABAA receptor-like genes. Mol Bio Evol 2007;24:599-610.
8. Anctil M. Chemical transmission in the sea anemone Nematostella vectensis: a genomic perspective. Comp Biochem Physiol Part D Genomics Proteomics 2009;4:268-89.

Epitope mapping of a cross-reactive monoclonal antibody against the factor H-binding protein of *Neisseria meningitidis*

A. Castello,[1] I. Costa,[1] I. Pernice,[1] R. Galbo,[1] O. Romeo,[1] D. Granoff,[2] C. Lo Passo[1]

[1]Department of Biological and Environmental Sciences, University of Messina, Italy; [2]Children's Hospital Oakland Research Institute, Oakland, CA, USA

Abstract

Neisseria meningitidis is an encapsulated gram-negative bacterium, major cause of bacterial meningitis and sepsi worldwide. Although polysaccharide-protein conjugated vaccines are available for the prevention of diseases caused by strains with group A, C, W-135 or Y capsules, no broadly protective vaccine is available against group B strains. The factor H binding protein (fHbp), a 27-kDa membrane-anchored lipoprotein of *Neisseria meningitidis,* is a promising vaccine candidate that elicits serum bactericidal antibodies in humans. The presence of factor H (fH) on the bacterial surface is critical to circumvent host defense while, in the absence of bound fH, the organism becomes susceptible to bacteriolysis. Based on sequence variability of the entire protein, fHbp has been divided into three variant groups or two sub-families. A panel of anti-fHbp mAbs has been produced from mice immunized with the 3 variants of fHbp and their epitopes were previously mapped, except for the mAb designated JAR36, a murine IgG mAb isolated from a mouse immunized with variant 3. In this study, we report epitope mapping of JAR36, this mAb cross-reacts with all fHbp sequences in V.2 and V.3 groups, binds to the bacterial surface and elicits complement-mediated bactericidal activity in combination with other anti-fHbp mAbs. We screened bacteriophage-displayed random peptide libraries to identify amino acid residues contributing to the JAR36 epitope. Mapping predictions were validated by constructing, through site-specific mutagenesis, corresponding rfHbps single-point variants, and analyzing their reactivity with the mAb.

Methods

The epitope recognized by JAR36 is located in the variable E (VE) segment of modular groups II, III, V, VI, VII in the C-terminal region of a chimeric *fHbp*. A multiple sequence alignment of the VE segment, comprising residues 186 to 255, from these *fHbp* variants.[1,2] Peptides binding to JAR36 mAb were selected by panning five phage libraries (pVIII-9aa, pVIII-9aa.cys, pVIII-12aa, pVIII-Cys.Cys, pVIII-15aa) constructed in the two-gene/phagemid vector pC89.[3] Mutants were constructed using the Phusion Site-Directed Mutagenesis Kit. Factor H binding protein was expressed from pET21b based plasmids in *E. coli* as described previously.[4,5]

Results

Forty-five positive clones were identified by screening phage libraries with JAR36. Among these clones there were 15 independent sequences. From the comparison of amino acid composition, obtained by characterization of the nucleotide sequence of the positive clones insert, it was not possible to identify a common consensus sequence among the peptides that reacted with JAR36, suggesting that the epitope is discontinuous. We hypothesized that the most abundant amino acids in the bound peptides might be important for the interaction between the immunogen and the mAb. Since JAR36 reacts with *fHbps* from variant groups 2 and 3, and more specifically with those sequences containing a variable E (VE) segment from lineage 2,[6,7] we focused our attention on the amino acids more frequently occurring in the peptide sequences, and located between positions 186 and 255 in the sequence of *fHbp* ID 28 (Figure 1). Consequently, we predicted a major contribution of the

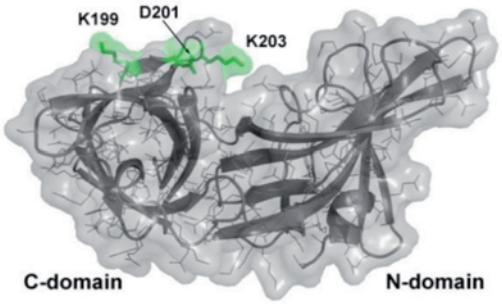

Figure 1. Location of residues predicted to affect binding of JAR36 mAb. A homology model of *fHbp* ID 28 was constructed using Swiss-Model[6] based on the atomic coordinates of the crystal structure of *fHbp* ID 1 (PDB accession number 3KVD).[7] The protein is represented with the C-terminal domain on the left and the *fH*-binding surface at the top. The locations of the three residues tested by site-specific mutagenesis are shown in green. The figure was generated by using PyMol (http://www.pymol.org).

Correspondence: Carla Lo Passo, Department of Biological and Environmental Sciences, University of Messina, Via F. Stagno D'Alcontres 31, 98166 Messina, Italy.
E-mail: clopasso@unime.it

Journal of Biological Research 2015; 88:5161

electrostatically charged residues aspartate and/or lysine to the JAR36 epitope. We examined the positions of relevant aspartate and lysine residues in a homology model of the *fHbp* ID 28 protein. We then substituted the residues K199, D201 and K203 with alanine and expressed and purified the *fHbp* mutants. Binding of JAR36 or human *fH* to site-specific mutants of *fHbp* ID 28 was measured by ELISA.

Conclusions

Three single amino acids positions composing the JAR36 epitope were predicted and the corresponding *fHbp* mutants were prepared. The K199A and K203A point mutants show decreased binding to human fH, but not to JAR36. The amino acid residue substitution, D201A in fHbp affects the binding of JAR36, but not that of human fH, thus Aspartate 201 is a necessary component of the JAR36 epitope.

References

1. Beernink PT, Granoff DM. The modular architecture of meningococcal factor H-binding protein. Microbiology 2009;155:2873-83.
2. Pajon R, Beernink PT, Harrison LH, Granoff DM. Frequency of factor H-binding protein modular groups and susceptibility to cross-reactive bactericidal activity in invasive meningococcal isolates. Vaccine 2010;28:2122-9.
3. Felici F, Castagnoli L, Musacchio A, et al. Selection of antibody ligands froma large library of oligopeptides expressed on a multivalent exposition vector. J Mol Biol 1991;222:301-10.
4. Masignani V, Comanducci M, Giuliani MM, et al. Vaccination against Neisseria meningitidis using three variants of the lipoprotein GNA1870. J Exp Med 2003;197:789-99.
5. Beernink PT, Granoff DM Bactericidal antibody responses induced by meningococcal recombinant chimeric factor H-binding protein vaccines. Infect Immun 2008;76:2568-75.
6. Schwede T, Kopp J, Guex N, Peitsch MC. SWISS-MODEL: an automated protein homology- modeling server. Nucleic Acids Res 2003;31:3381-5.
7. Cendron L, Veggi D, Girardi E, Zanotti G Structure of the uncomplexed Neisseria meningitidis factor H-binding protein fHbp (rLP2086). Acta Crystallogr Sect F Struct Biol Cryst Commun 2011;67:531-5.

Oxidative stress responses in early life stages of common carp (*Cyprinus carpio*) after subchronic exposure to NeeMazal T/S (azadirachtin 10 g/L)

F. Casuscelli di Tocco,[1] J. Blahova,[2] L. Plhalova,[2] D. Zivna,[2] M. Sevcikova,[2] L. Chromcova,[2] L. Divisova,[2] C. Faggio,[1] Z. Svobodova[2]

[1]Department of Biological and Environmental Sciences, University of Messina, Italy; [2]Department of Veterinary Public Health and Animal Welfare, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

Increased use of chemical pesticides results in the excess inflow of toxic chemicals into the aquatic ecosystem and may affect aquatic flora and fauna. Recent emphasis is on the use of natural pesticides, which are usually of plant origin. Use of plant based pesticides is generally less harmful and more ecofriendly. Plant based pesticides contain easily biodegradable molecules which are more target specific than the highly persistent broad-spectrum synthetic chemical moieties.[1] Azadirachtin is an active compound extracted from the neem tree (*Azadirachta indica* A. Juss) grown widely in Africa and Asia. Its anti-insecticidal, antiviral, antibacterial, and antifungal properties have been known for long time.[2] Azadirachtin is the active substance of insecticide formulation NeemAzal T/S (concentration of active ingredients, 10 g/L), which is used to control whitefly, leaf miners, and other pests including pear psylla. In foreign countries, it has been generally distributed and widely used for a long period of time. Nevertheless, NeemAzal T/S has been registered in the Czech Republic since 2010. NeemAzal T/S is classified as highly toxic to aquatic animals.

The objective of our study was to evaluate changes in antioxidant defense enzymes and detoxifying system in early life stages of common carp after exposure to NeemAzal T/S. Subchronic toxic effects on embryos and larvae were investigated during a 31-day embryolarval toxicity test, which was carried out using a modified protocol according to the OECD guideline 210 (Fish, Early-Life Stage Toxicity test). Testing solutions were prepared from NeemAzal T/S with the nominal concentration of azadirachtin: 30, 100, 300 and 600 µg/L. Twenty-four hours after fertilization, one hundred fertilized eggs were separated and randomly distributed into crystallization dishes containing one of the four concentrations of azadirachtin solution, or a control dishes (azadirachtin-free tap water). The experiment was conducted in triplicate (a total of 300 fertilized eggs for each concentration and control). A semistatic method was used and the testing solution was replaced twice daily. During the test, larvae were fed freshly hatched *Artemia salina ad libitium* twice a day. The temperature, pH, and oxygen saturation were recorded daily. At the end of the experiment, the fish were killed, immediately frozen, and stored at -85°C until analyses of biomarkers. Whole body samples were weighed and homogenised using phosphate buffer. Supernatant fraction of homogenate was used for determination of activity of glutathione S-transferase (GST),[3] glutathione reductase (GR),[4] glutathione peroxidase (GPx)[5] and catalase (CAT).[6] Protein concentration was determined by Bicinchoninic Acid Protein Essay Kit (Sigma-Aldrich). Statistical analysis was performed using Statistica 8.0 for Windows software. Biomarkers were tested for normal distribution and after testing of homogeneity of variance across groups, an analysis of variance (one-way ANOVA) was used. The differences among groups were assessed with the Tukey-HSD test.

Effects of subchronic exposure to NeemAzal T/S on selected oxidative stress indices and activity of detoxifying enzyme are presented in Table 1. In all experimental groups, increases of the GR, GPx and GST activities were observed compared to the control group but only in the groups exposed to azadirachtin at 300 and 600 g/L the level reached significance ($P<0.05$). In case of CAT activity we did not obtain differences among groups.

In our experiment, we found higher activities in almost all enzymes (GR, GPx and GST) in all experimental groups compared to the control group. An increase in antioxidant enzymes contributes to the elimination of reactive oxygen species, which are induced by pesticide exposure. The increase in GST activity is connected with activation of detoxifying system after NeemAzal T/S exposure. Although botanical pesticides are being considered as less toxic and safe, our results indicate that azadirachtin may affect antioxidant defence system and detoxifying ability of fish organism. Negative effects of azadirachtin were also reported by other toxicology studies. Kumar and colleagues[7] observed morphological changes in vital organs such as gill of *Heteropneustes fossilis* after short- and long-term exposure to purified neem extract. Winkaler and colleagues[8] reported effects of acute lethal and sublethal exposure to neem leaf extract on the neotropical freshwater fish *Prochilodus lineaus*. Plasma glucose levels were higher in fish exposed to neem extract relative to controls, indicating a typical stress response. Neem extract did not interfere with osmoregulation capacities of the fish, as plasma sodium, chloride, total protein, and osmolarity did not change. It was shown to affect the antioxidant defence system of *P. lineatus*, as there was a decrease in liver CAT activity at all neem concentrations, and the detoxifying enzyme GST was activated in fish exposed to the highest concentration. At all concentrations, exposed fish exhibited damaged gill and kidney tissue.

Correspondence: Caterina Faggio, Department of Biological and Environmental Sciences, University of Messina, viale F. Stagno D'Alcontres 31, 98166 Messina, Italy.
E-mail: caterina.faggio@unime.it

Acknowledgements: this research was supported by GACR P502/12/P163.

Table 1. Results of oxidative stress indices (mean±SEM). Significant differences (P<0.05) among groups are indicated by different alphabetic superscipts.

Concentrations of azadirachtin (µg/L)	GPx (nmol/min/mg protein)	GR (nmol/min/mg protein)	GST (nmol/min/mg protein)	CAT (µmol/min/mg protein)
0 (control)	77.8±6.0^{a}	7.7±0.3^{a}	97.8±2.1^{a}	25.0±1.0^{a}
30	84.3±7.1^{ac}	8.2±0.4^{ab}	104.0±7.9^{ab}	24.5±1.4^{a}
100	84.7±4.2^{ac}	8.3±0.5^{ab}	100.4±1.6^{ab}	23.6±1.3^{a}
300	108.1±5.1^{b}	10.1±0.2^{b}	109.1±1.9^{b}	27.4±1.3^{a}
600	99.46±3.0^{bc}	10.1±0.4^{b}	108.5±2.6^{b}	23.2±0.9^{a}

GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione S-transferase; CAT, catalase.

References

1. Stalin SI, Koruna S, Das SSM. A comparative study on the toxicity of a synthetic pyrethroid deltamethrin and a neem based pesticide, azadirachtin to Poecilla reticulata Peters 1859 (Cyprinodontiformes: Poeciliidae). Turk J Fish Aquat Sci 2008;8:1-5.
2. Isman MB, Koul O, Luczyski A, Kaminski J. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J Agric Food Chem 1990;38:1406-11.
3. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. First enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249:7130-9.
4. Carlberg I, Mannervik B. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem 1975;250:5475-80.
5. Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol 1984;105:114-21.
6. Aebi H. Catalase in vitro. Method Enzymol 1984;105:121-6.
7. Kumar A, Prasad MR, Srivastava K, Tripathi S, Srivastav AK. Branchial histopathological study of catfish Heteropneustes fossilis following exposure to purified neem extract, azadirachtin. World J Zool 2010;5:2039-43.
8. Winkaler EU, Santos TRM, Machalo-Neto JG, Martinez CBR. Acute lethal and sublethal effects of neem leaf extract on the neotropical freshwater fish Prochilodus lineatus. Comp Biochem Physiol 2007; 145:236-44.

Spectroscopic characterization and *in vitro* assay on human blood of novel porphyrin derivatives

F. Ceraolo,[1] M. Vazzana,[1] M.A. Castriciano,[2] A. Mazzaglia,[2] R. Zagami,[3] A. Romeo,[3] C. Faggio[1]

[1]Department of Biological and Environmental Sciences, University of Messina; [2]CNR-ISMN-Institute for the Study of Nanostructured Materials, Department of Chemical Sciences, University of Messina; [3]Department of Chemical Sciences, University of Messina, Italy

Porphyrins are an important class of natural and artificial pigments which play an important role in largely different area of both fundamental and technological interest. In particular, porphyrin metal derivatives have been exploited as models for enzymes and artificial blood. Charged porphyrins are able to interact with several relevant biomolecules, *i.e.*, nucleic acids, polypeptides, and proteins. This property together with their ability to localize into tumor cells and to photosensitize the production of singlet oxygen led to the development of several compounds actually in use or under investigation for photodynamic therapy applications (PDT).[1] In this approach visible light is used to active a photosensitizer, generating the highly reactive singlet molecular oxygen. To date, a great variety of porphyrins have been extensively studied. Interestingly, quite recently, meso-tetrakis(4-carboxyphenyl) porphyrin (TPPC) and its derivatives have been exploited as a marker for the rapid detection of tumor cells by fluorescence imaging. It is important to note that many of the physicochemical properties of this class of pigments, and in particular the electronic absorption and the luminescence, are strictly dependent on their aggregation state. A common feature of these molecules is their propensity to interact to form dimers, oligomers, or more extended aggregates. To develop efficient systems for biomedical applications or for PDT (in which aggregation should be prevented) or to stabilize monomeric porphyrins in a very water-soluble form, novel systems based on biocompatible delivery systems are highly desirable. In this framework, recently some of us reported on the employment of biocompatible amino-terminated polypropylene or poly(ethylene oxide)s generally termed as Jeffamines, to prevent porphyrin aggregation, allowing to reach millimolar concentration of TPPC in a monomeric form in solution.[2] In biological media, cell membranes seem to be important targets for many antineoplastic photosensitizer agents. Red blood cells have been often used for *in vitro* PDT studies. Here we report on two different porphyrin derivatives, TPPC-Jeff and ZnTPPC-Jeff. In Figure 1 is reported the structure of TPPC-Jeff. Photodynamic action was then evaluated *in vitro* using human red blood (HRB) cells under different conditions to obtain information about the effect produced by these porphyrin derivatives upon irradiation. These novel compounds were tested on human red blood cells (RBCs), with the purpose to see an haemolytic effect on the erythrocytes after exposure to Vis irradiation and overnight incubation. Human blood samples were collected from four healthy donators, were drawn into syringes filled with sodium citrate, as an anticoagulant and were used within 24 h after bleeding. Each, of the two compounds, was dissolved in a buffer, a saline solution at pH 7.4 with the following composition (mM): 125 NaCl, 5 KCl, 1 $MgSO_4$, 32 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES), 5 D-(+)-glucose, 1 $CaCl_2$; pH 7.4. The samples (HBR cells, saline solution and molecule) were placed in glass flasks (10 mL) and were exposed to 30 minutes to Vis irradiation (halogen lamp with a light dose of 5 joule/cm^2). The molecules were tested at four different concentrations spanning from 5.10^{-6} M up to 10^{-4} M. In all studies, using HRB cells, control experiments were carried out without porphyrin compounds in presence and absence of Vis irradiation.

We used an hemolysis test to see the hemolytic effects of the porphyrin compounds, that evaluates hemoglobin release in the plasma

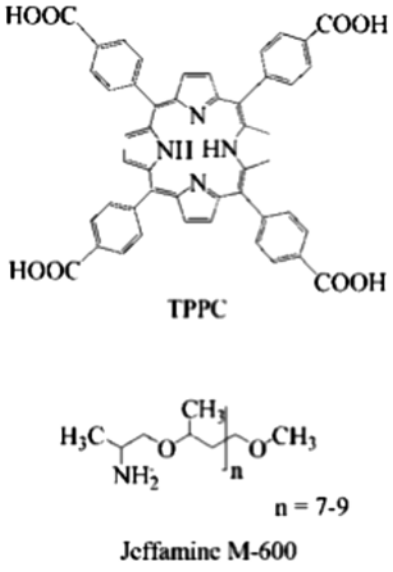

Figure 1. Structure of the TPPC-Jeff.

Correspondence: Caterina Faggio, Department of Biological and Environmental Sciences, University of Messina, viale F. Stagno D'Alcontres 31, 98166 Messina, Italy.
E-mail: caterina.faggio@unime.it

Journal of Biological Research 2015; 88:5161

following molecule and irradiation exposure.[3] After overnight incubation at 37°C, the tubes were centrifuged (10 minutes at 1500 rpm at 4°C) and the supernatants were determined photometrically with a colorimeter and after with a spectrophotometer at the absorption of hemoglobin (540 nm). The spectroscopic measurements of haemoglobin released are much easier and faster to carry out than cell staining and counting.[4] The absorption of the supernatant of erythrocytes lysed in distilled water was defined as 100% haemolysis. The molecules under study induce hemolysis in human erythrocytes at the concentrations tested and then overnight incubation, as show in Table 1. The concentrations 5.10^{-6} M and 10^{-4} M are respectively one too low and the other too high to see a reliable hemolytic effect. While we observed that TPPC-Jeff induce an hemolytic effect at the concentration of 5.10^{-5} M, and the Zn TPPC-Jeff induce the release of hemoglobin at the concentration of 10^{-5} M. We have seen that Vis irradiation does not increase the efficacy of the compounds in inducing hemolysis.

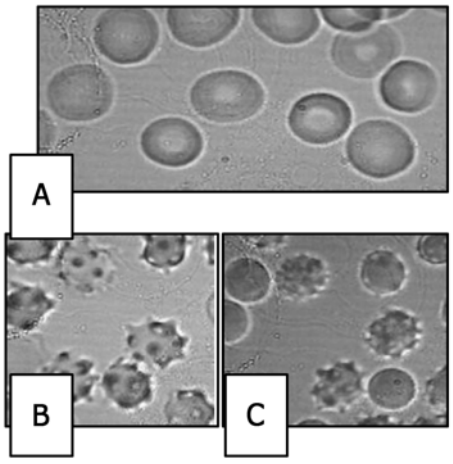

Figure 2. Photograph showing morphology of HRB cells (A) control without tretracycline and irradiated for 30 min; (B) TPPC-Jeff and irradiated for 30 min; (C) Zn TPPC-Jeff and irradiated for 30 min.

Table 1. Percentage of hemolysis by compounds TPPC-Jeff (5.10^{-5} M), Zn TPPC-Jeff (10^{-5} M) osmolarity (mOsm/kg).

Compounds	300	200	150	100	0
Control	0	1	2	18	100
TPPC-Jeff	12	11	12	30	100
ZnTPPC-Jeff	17	16	23	100	100

Then we assayed the morphology of erythrocytes by an optical microscope after incubation with porphyrin compounds, 30 minutes of irradiation and overnight incubation and the result was that they lost their normal biconcave profile and presented a spiny configuration with blebs in their surfaces. A normal profile was observed in the control. Representative results are show in Figure 2. HRB cells obtained from four subjects were used to assess an hemolytic activity of porphyrins. Results suggest that all the molecules under study show an hemolytic effect. Further studies are required to optimize potential therapeutic dosing strategies to inform and encourage clinical trial design.

References

1. Pandey RK, Zheng G. Porphyrins as photosensitizers in photodynamic therapy. In: Kadish KM, Smith KM, Guilard R (eds.), The porphyrin handbook. Academic Press: New York; 2000. p. 157.
2. Castriciano MA, Romeo A, Angelini N, et al. Structural features of meso-tetrakis(4-carboxyphenyl) porphyrin interacting with amino-terminated poly (propylene oxide), Macromolecules 2006;39:5489-96.
3. Bonaccorsi P, Aversa MC, Barattucci A, et al. Sulfenic acid-derived glycoconjugated dusulfides and sulfoxides: a biological evaluation on human red blood cells. Journal of Sulfur Chemistry 2013 (in press).
4. Ochoa A.L., Tempesti T.C., Spesia M.B., Milanesio E., Durantini E.N. Synthesis and photodynamic properties of adamantylethoxy Zn (II) phthalocyanine derivatives in different media and in human red blood cells. European Journal of Medicinal Chemistry 2012;20:280-7.

Mitochondrial mass, distribution and activity during sea urchin oogenesis

M. Agnello, A.M. Rinaldi, R. Chiarelli, L. Bosco, G. Morici, M.C. Roccheri

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

The sea urchin egg is a favourite model for studies of the molecular biology and physiology of fertilization and early development, yet we know sparingly little of its oocytes and of mitochondria behaviour during oogenesis. The process of oogenesis in most echinoderms is asynchronous so each ovary lobe has hundreds of oocytes at all stages of development. At the beginning of oogenesis, the oocyte is about 10 μm in diameter. During the vitellogenic phase of oogenesis, the oocyte accumulates yolk proteins and grows to ten times their original size to 80 to 100 μm in sea urchins. The oocyte, arrested at the prophase of the first meiotic division, is apparent with its large nucleus, the germinal vesicle (GV), containing a prominent nucleolus. Echinoid (such as sea urchin) and Holothurian oocytes complete meiotic maturation prior to fertilization, distinct from other echinoderms and almost all others animals. As maturation progresses, it occurs the GV breaks down (GVBD). These eggs may then be stored for weeks to months within the female before they are spawned, and the proportion of eggs in the ovary increases from early to late season, as the numbers of oocytes decline.[1]

Mitochondria, generally known as the powerhouses of eukaryotic cells, play a primary role in cellular energetic metabolism, homeostasis and death. These organelles, with their multicopy genome maternally inherited, are directly involved at several levels in the reproductive process since their functional status influences the quality of oocytes and contributes to the process of fertilization and embryonic development.

It has been demonstrated that the number of maternal mitochondria is sufficient to support development until late stages without new synthesis of mitochondrial DNA or production of new organelles.[2] During embryogenesis mitochondrial mass does not change, whereas mitochondrial respiration increases.[3] The behaviour of these organelles during oogenesis remains at moment unclear.

In the present paper we studied, by Confocal Laser Scanning Microscopy tecnologies (CLSM), the mass and distribution, the activity and the DNA content of sea urchin *Paracentrotus lividus* mitochondria during oogenesis, by *in vivo* incubating oocytes of different size with cell-permeant probes specific for mitochondria and for DNA and by immunodetection of hsp60 chaperonine, a well known mitochondrial marker.

In particular the oocytes were grouped in six classes: <10, 20/30, 40/50, 60/70, 80/90 μm, and 90 μm ovulated egg, on the base of diameters. Microscopic observations were performed capturing 2 μm thick layers of oocytes. Of the several thousands oocytes we observed, 20 for each different oogenesis stage were analyzed and processed. In order to interpret results and to draw unequivocal conclusions, we measured by IMAGE J software analysis the intensity values of fluorescent signals, as suggested in Agnello and colleagues 2008.[4]

The mitochondria of oocytes with a diameter between 20 and 70 μm, appeared to give rise to clusters that disappear in that of 80 μm. In the oocytes between 60 and 90 μm the red fluorescence seems to be more evident around the germinal vesicle (the merge tends to red), suggesting an increasing oxidative phosphorylation activity.

In the ovulated eggs, red and green fluorescence are uniformly distributed suggesting that mitochondria are dispersed in the cytoplasm. In addition the merge of green and red colours shows that the whole mitochondrial population is consuming oxygen at the same level (the resulting colours tends to yellow; Figure 1).

In order to calculate the total mitochondrial mass and activity we integrated the values of pixel intensities for all captured sections and used the arithmetic means to draw a statistical analysis. Results suggest a parallel rise of mitochondrial mass and activity, suggesting that the amount and activity of organelles change remarkably during oogenesis. Results also suggest that mitochondria are actively duplicating and that mitochondrial DNA is replicating during the different oogenesis phases. It is noteworthy that around the germinal vesicle, especially in the larger oocytes, next to the germinal vesicle breakdown, the organelles are more active in oxygen consumption, probably due to the major energetic needing in this key moment of gametogenesis.

Correspondence: Maria Carmela Roccheri, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze, Ed. 16, 90127 Palermo, Italy.
E-mail: maria.roccheri@unipa.it

Journal of Biological Research 2015; 88:5161

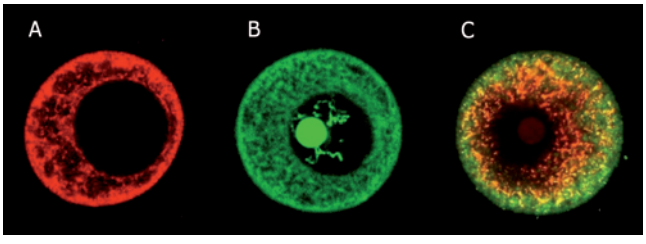

Figure 1. Distribution of hsp60 protein detected by immunofluorescence analysis (A), the mitochondrial and genomic DNA, after *in vivo* incubation with PicoGreen probe (B) and the merge of green and red fluorescence signal, respectively due to mitochondrial mass and activity, after *in vivo* incubation with Mitotraker Green and Orange (C). The size of the oocytes reported is 80 μm.

References

1. Wessel GM, Voronina E, Brooks JM. Obtaining and handling echinoderm oocytes. In: Methods in cell biology. Amterdam: Elsevier; 2004. pp. 87-114.
2. Matsumoto L, Kasamatsu H, Piko L, Vinograd J. Mitochondrial DNA replication in sea urchin oocytes. J Cell Biol 1974;63:146-59.
3. Morici G, Agnello M, Spagnolo F, et al. Confocal microscopy study of the distribution, content and activity of mitochondria during *Paracentrotus lividus* development. J Microscopy 2007;228:165-73.
4. Agnello M, Morici G, Rinaldi AM. A method for measuring mitochondrial mass and activity. Cytotechnology 2008;56:145-9.

Obtaining mesenchymal stem cells from adipose tissue of murine origin: experimental study

R. Altomare,[1] A. Abruzzo,[1] V. Palumbo,[1,2] G. Damiano,[2] G. Spinelli,[2] A. Bruno,[2] L. Cicero,[3] G. Cassata,[3] G. Tomasello,[2] F. Cacciabaudo,[1] M.C. Gioviale,[4] F. Damiani,[1,4] A.I. Lo Monte[1,2,4]

[1]School in Surgical Biotechnology and Regenerative Medicine, University of Palermo; [2]Department of Surgical, Oncological and Stomatological Sciences, University of Palermo; [3]The Institute for Experimental Veterinary Medicine of Sicily A. Mirri, Palermo, Italy; [4]P. Giaccone Hospital, University of Palermo, Italy

Stem cells have a key role in regenerative medicine and tissue engineering. Although not immortal, they are able to expand manyfold in culture retaining at the same time their growth and multilineage potential. They also show a migratory capacity when transplanted systemically in animal model with injuries.

Thanks to their properties and their plasticity stem cells are of great importance since they can be used as a tool for repair damaged tissues and organs.

Mesenchymal stem cells, in particular, have the ability to differentiate into lineages of mesodermal tissues, such as skeletal muscle, bone, tendons, cartilage, and fat under appropriate culturing conditions.

Recent evidence suggest that the adipose tissue is a promising source of mesenchymal stem cells attracting the interest of researchers and clinicians. It is rich of pluripotent stromal cells , available in large amounts and more readily accessible than bone marrow. Furthermore, comparative analysis of mesenchymal stem cells of bone marrow and adipose tissue show that cells are not different regarding morphology, immune phenotype, success rate of isolating and differentiation ability.

Our experience at the Experimental Zooprophylactic Institute of Sicily A. Mirri allows us to define a protocol for stem cells isolation of murine origin.

We used 6 Winstar breed male rats whose average weight was 350g.

All animals were sedated with an intramuscular injection of midazolam and anesthesia was maintained with isoflurane and oxygen gas mixture administered with a mask.

The adipose tissue has been taken from the root of the animal's thigh with a small incision. Different steps are needed for processing and digesting the tissue. First it is washed several times in a solution enriched of antibiotics and then is mechanically fragmented. The homogenate is therefore digested enzymatically under permanent shaking.

We obtain an heterogeneous population of cells that were subsequently selected through the plastic adhesion. These cells are able to grow and proliferate and show all the characteristics typical of stem cells. In conclusion, we report a multistep and reproducible technique for providing a substantial number of mesenchymal stem cells and for maintaining them in culture.

Correspondence: Roberta Altomare, School in Surgical Biotechnology and Regenerative Medicine, University of Palermo, via del Vespro 131, 90127 Palermo, Italy.
E-mail: roberta.altomare@unipa.it

Role of ERK1/2 protein in the regulation of *Herpes simplex* virus type I replicative cycle

I. Colao,[1] G. Siracusano,[1] M.G. Ceraolo,[1] A.Venuti,[2] H. Veijo,[3] A. Mastino,[1] M.T. Sciortino[1]

[1]Department of Biological and Environmental Sciences, University of Messina; [2]Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy; [3]Department of Virology, University of Turku, Finland

Introduction and Objectives

Herpes simplex virus type 1 (HSV-1) is a double stranded DNA (dsDNA) virus that causes a variety of infections in humans.[1] HSV-1, as many DNA viruses, has developed different strategies during the evolution to modify the cellular environment in favor of its replication. Different aspects of HSV-1 biology render this virus a good model to study the complexity of virus-host cell interactions. The eukaryotic cells, indeed, respond to external stimuli through the activation of different signal pathways, as the Ras/Raf/MEK/ERK signal pathway. Among these protein kinases, the extracellular-signal-regulated-kinases (ERK) have proven critical in the control of the progression G1/S that involved specific regulator proteins, such as cyclins and cyclin-dependent kinases (CDKs).[2,3] That HSV-1 infection requires involvement of ERK1/2 and mitogen activated proteins kinases (MAPKs) signal pathway and controls cell cycle proteins is already known.[4-9] In fact, the activity of CDK involved in the transition from late G1 to early S phase seems to be required for viral DNA transcription and replication. However, the evidence for the overall understanding of networks and gene products involved in these interaction requires further investigations. Based on these knowledge the current work was focused to study the activity of ERK1/2 protein during viral replication and the correlation between ERK protein recruitment and G1/S phases regulation by HSV-1 infection.

Materials and Methods

Western Blot analysis was used to evaluate nuclear and cytoplasmic protein accumulations. The MAPK/MEK-ERK pathway was modulated using the inhibitor U0126 to study replication in HEp-2 (human larynx epidermoid carcinoma cell line). A stably transfected cell line was derived from wild type (wt) HEp-2, by transfection of plasmid coding for dominant negative form of ERK protein (HEp-dnERK). Standard Plaque Assay was done on VERO cells. Immunofluorescence assay and quantitative Real Time PCR were used to evaluate the levels of viral and cellular gene transcription and viral DNA synthesis.

Correspondence: Ivana Colao, Department of Biological and Environmental Sciences, University of Messina, viale F. Stagno d'Alcontres, 98166 Messina, Italy.
Tel: +39.349.7844545.
E-mail: icolao@unime.it

Journal of Biological Research 2015; 88:5161

Results and Discussion

We have analyzed the activation of ERK1/2 protein during wt HSV-1 infection in HEp-2 wt cell line. HSV-1 leads to the activation of ERK1/2 protein during the first phases of infection, and subsequent decrease during late phases compared to uninfected cells, suggesting involvement of ERK1/2 activity during infection. HSV-1 replication was studied in wt HEp-2 cells where ERK1/2 activity was chemically inhibited. The data showed a defect in viral progeny production in treated and infected cells as compared with non-treated and infected cells. These data were confirmed by the differences in the accumulation of ICP0 (immediate early) and Us11 (late) viral proteins. Moreover, we evaluated the phosphorylated forms of key regulators of G1/S progression, such as cyclin E and CDK2 proteins in presence or in absence of U0126. The results demonstrated that the treatment inhibits the accumulation of cyclin E and CDK2 proteins. These results were further confirmed by using HEp-dnERK cell lines. In this cell system HSV-1 replication was compromized compared with parental cell lines. Indeed, using q-PCR, viral DNA the cellular genes CDK2 and cyclin E, and the viral immediately early gene (ICP0) and the late gene, (gB) were evaluated in HEp-dnERK infected by wt HSV-1 compared to control. A decrease was observed in viral DNA synthesis, as well as in cellular gene transcripts, in cells where ERK1/2 activity was compromised, demonstrating that MAPK-ER proteins plays a fundamental role during HSV-1 replication. However, further investigations are necessary.

Conclusions

The new information obtained could be contribute to development of new pro-host tools that would be useful to set up effective prevention strategies, such as therapeudical approaches for severe HSV-associated infections. Because the Raf/Ras/MEK/ERK pathway is modified in 60% of solid tumors, understanding the interactions between HSV-1 and this pathway could contribute to the design of HSV-1-based oncolytic vectors.

References

1. Whitley RJ, Roizman B. Herpes simplex virus infections. Lancet 2001;357:1513-8.
2. Chambard JC, Lefloch R, Pouysseguer J, Lenormand P. ERK implication in cell cycle regulation. Biochim Biophys Acta 2007;1773: 1299-1310.
3. Villanueva J, Yung Y, Walker JL, et al. ERK activity and G1 phase progression: identifying dispensable versus essential activities and primary versus secondary targets. Mol Biol Cell 2007;18:1457-63.
4. Farassati F, Lee PW. Ras signalling pathway: a gateway for HSV-1 infection. Sci World J 2003;3:533-5.
5. Torres NI, Castilla V, Bruttomesso AC, et al. In vitro antiviral activity of dehydroepiandrosterone, 17 synthetic analogs and ERK modulators against herpes simplex virus type 1. Antiviral Res 2012;95:37-48.
6. Chuluunbaatar U, Roller R, Mohr I. Suppression of extracellular signal-regulated kinase activity in HSV-1 infected cells by the Us3 protein kinase. J Virol 2012;86:7771-6.
7. Kalamvoki M, Roizman B. Interwoven roles of cyclin D3 and cdk4 recruited by ICP0 and ICP4 in the expression of herpes simplex virus genes. J Virol 2010;84:9709-17.
8. Ehmann GL, McLean TI, Bachenheimer SL. Herpes simplex virus type 1 infection imposes a G(1)/S block in asynchronously growing cells and prevents G(1) entry in quiescent cells. Virology 2000;267: 335-49.
9. Orlando JS, Astor TL, Rundle SA, Schaffer PA. The products of the herpes simplex virus type 1 immediate-early US1/US1.5 genes downregulate levels of S-phase-specific cyclins and facilitate virus replication in S-phase Vero cells. J Virol 2006;80:4005-16.

Effect of conditioned media from osteo- and adipo-differentiating mesenchymal stem cells on triple negative MDA-MB231 breast cancer cells

M. Librizzi,[1] A. Longo,[1] O. Consiglio,[1] E. Tobiasch,[2] C. Luparello[1]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy; [2]Department of Natural Sciences, University of Applied Sciences, Bonn-Rhein-Sieg, Germany

It is know that mesenchymal stem cells (MSCs) actively secrete multiple biologically-active factors during their process of differentiation which gives rise to a variey of cytotypes including bone and fat cells. It is also acknowledged that the chemokines secreted throughout MSC differentiation may play an important role in the development and growth of tumor cells, although literature data appear somewhat indeterminate due to the contradictory evidence often found.[1]

The purpose of this study was to evaluate the effect of conditioned media (CMs) from MSCs, cultured for 7, 14, 21 and 28 days in osteo-, adipo-differentiating and undifferentiated conditions, on MDA-MB231 breast cancer cells, an *in vitro* model system derived from a triple-negative breast cancer (TNBC). MTT assay showed that the CMs collected after 28 days of both osteo- and adipo-differentiation induced growth inhibition on MDA-MB231 cells after 24 h of incubation. In light of such evidence, these CMs were used to treat cells and perform cytofluorimetric assays to better evaluate their biological effects on viability/proliferation, cell cycle progression, apoptosis/autophagy induction and mitochondrial activity/reactive oxygen species (ROS) accumulation of MDA-MB231 cells.

The most interesting results regard the ability of CMs from osteo-differentiating MSC to induce an alteration of cell proliferation with an arrest in the G_2/M transition phase of the cycle coupled to both apoptotic and autophagic promotion. No accumulation of ROS and impairment of mitochondrial respiration was observed at the end of treatment. On the other hand, preliminary indications suggest that the CMs isolated from adipo-differentiating MSCs have different effect from those obtained by osteo-differentiating cultures, being the lethality unlinked to apoptosis and autophagy, and thereby prompting to get more insight into the anti-TNBC activity shown by the different CMs at the molecular level.

References

1. Alan PM, Fiachra TM, Roisin MD, et al. Mesenchymal stem cell secretion of chemokines during differentiation into osteoblasts, and their potential role in mediating interaction with breast cancer cells. Int J Cancer 2009;124:326-32.

Correspondence: Claudio Luparello, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze Ed.16, 90127 Palermo, Italy.
E-mail: claudio.luparello@unipa.it

Funding: this work was supported by University of Palermo (R.S. ex60% and FFR 2013) and Progetto Vigoni 2011 for Claudio Luparello; BMBF, AdiPaD, 1720X06, FHprofUnt, FKZ: 03FH012PB2; FH-Extra, FKZ: z1112fh012; DAAD, PPP Vigoni, FKZ: 54669218; BMBF-AIF, FKZ: 1720X06; and Geräteprogramm, Fachhochschulen NRW 2008 for Edda Tobiasch.

Effect of copper chelation on hepcidin expression in the human hepatoma cell line, HepG2

L.M. Di Bella, M.R. Felice

Department of Biological and Environmental Sciences, University of Messina, Italy

Hepcidin, a peptide hormone produced mainly by the liver,[1] has an essential role in iron homeostasis. It is produced as prepropeptide of 84 amino acids and, after two consecutive cleavage, a form of 25 amino acids is secreted in the bloodstreem.[2-4] Hepcidin is able to control the body iron concentration by the binding to the only known protein involed in iron export from the cells, ferroportin, that is present on the basolateral membrane of enterocytes, on macrophages, hepatocytes and other cells types.[5,6] After the binding, ferroportin is degraded and the iron uptake by the diet and iron recycling from senescent red blood cells, is inhibited.[7] View the important role of hepcidin in iron metabolism, its expression is strictly regulated; in fact, dysfunctions or mutations that modify its expression, lead to different pathologies, such as hemochromatosis and anemia.[8-10] Some crystallographic studies have demonstrated that an other important metal for the life of all the organisms, copper, could be important for hepcidin funtion; in fact, the presence of copper in solution can influence the aggregation state of hepcidin and studies in which a cell line was used as model system, have highlighted the importance of this element in the capacity of hepcidin to induce ferroprtin degradation.[11,12] The link between copper and iron is demonstarated also by an other important plasma protein, ceruloplasmin, a ferroxidase enzyme that contain copper in the catalytic site and is responsable of the transport of 90% of plasma copper[13] Different studies have demonstrated that animals fed with a copper deficient diet, have low levels of plasma ceruloplasmin and, its characteristic oxidase activity, is strongly reduced;[14,15] this kind of situation is normally present in Wilson disease patients, in which a defect in copper transport in the Golgi, is cause of the lack of plasma ceruloplasmin.[16] To better understand if the copper has a role on hepcidin expression, we have used a human hepatoma cell line, HepG2, to investigate the influence of copper chelation and inflammation, by the use of the citokine interleukin-6,[17] on the level of hepcidin expression. To corroborate our results, we have also studied ceruloplasmin expression. Our results have demonstarted that the use of the chelation substrate, Bathocuproinedisulfonic acid (BCS) has a slight effect on ceruloplasmin mRNA levels, both in normal and stimulated IL-6 cells, while the intracellular level of protein is markedly reduced when BCS is added in both conditions. The inhibitory effects is more evident if the secreted form of the protein is considered; in fact, western blot and in gel oxidase activity analysis, have shown a disappearence of the protein and of its activity in the growth cellular media, results coherent with bibliographic data. The effect on hepcidin is also evident. Even if no statistical significative differences were found at mRNA level, the effect on the concentration of the intracellular prepropeptide is more evident, especially when the citokine and BCS are used togheter. The presence of both substrates leads to a decrease of the protein level when it is compared to the use of interleukin-6 alone and this effect was more evident when the concentration of the peptide in the cellular growth media was assayed. Our results demonstrate that a copper deficiency could negatively influence hepcidin expression even if other studies are necessary to better understand this mechanism.

Correspondence: Maria Rosa Felice, Department of Biological and Environmental Sciences, University of Messina, via Stagno D'Alcontres 31, 98166 Messina, Italy.
E-mail: mrfelice@unime.it

Journal of Biological Research 2015; 88:5161

References

1. Krause A, Neitz S, Mägert H, et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett 2000;480:147-50.
2. Pigeon C, Ilyin G, Courselaud B, et al. A new mouse liver- specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem 2001;276:7811-9.
3. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 2001; 276:7806.
4. Valore EV, Ganz T. Posttranslational processing of hepcidin in human hepatocytes is mediated by the prohormone convertase furin. Blood Cells Mol Dis 2008;40:132-8.
5. Donovan A, Lima CA, Pinkus JL, et al. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab 2005;1:191-200.
6. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of mammalian iron metabolism. Cell 2010;142:24-38.
7. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004;306:2090-3.
8. Lanzara C, Roetto A, Daraio F, et al. Spectrum of hemojuvelin gene mutations in 1q-linked juvenile hemochromatosis. Blood 2004;103:4317-21.
9. Lee P, Peng H, Gelbart T, Beutler E. The IL-6- and lipopolysaccharide-induced transcription of hepcidin in HFE-, transferrin receptor 2-, and beta 2-microglobulin-deficient hepatocytes. P Natl Acad Sci USA 2004;101:9263-5.

10. Kearney SL, Nemeth E, Neufeld EJ, et al. Urinary hepcidin in congenital chronic anemias. Pediatr Blood Cancer 2007;48:57-63.
11. Hunter HN, Fulton DB, Ganz T, Vogel HJ. The solution structure of human hepcidin, a peptide hormone with antimicrobial activity that is involved in iron uptake and hereditary hemochro matosis J Biol Chem 2002;277:37597-603.
12. Melino S, Garlando L, Patamia M, et al. Metal-binding site is present in the amino terminal region of the bioactive iron regulator hepcidin-25. J Pept Res 2005;66(Suppl.1):65-71.
13. Meyer LA, Durley AP, Prohaska JR, Harris ZL. Copper transport and metabolism are normal in aceruloplasminemic mice. J Biol Chem 2001;276:36857-61.
14. Pyatskowit JW, Prohaska JR. Copper deficient rats and mice both develop anemia but only rats have lower plasma and brain iron levels. Comp Biochem Physiol C Toxicol Pharmacol. 2008;147:316-23.
15. Broderius M, Mostad E, Wendroth K, Prohaska JR. Levels of plasma ceruloplasmin protein are markedly lower following dietary copper deficiency in rodents. Comp Biochem Physiol C Toxicol Pharmacol 2010;151:473-9.
16. Matsuda I, Pearson T, Holtzman NA. Determination of apoceruloplasmin by radioimmunoassay in nutritional copper deficiency, Menkes' kinky hair syndrome, Wilson's disease, and umbilical cord blood. Pediatr Res 1974;8:821-4.
17. Nemeth E, Rivera S, Gabayan V, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 2004;113:1271-6.

Total internal reflection fluorescence microscopy as a powerful tool to follow dynamic events at the cell membrane

E.S. Di Cairano, S. Moretti, F. Daniele, V.F. Sacchi, C. Perego

Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy

Cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signaling. Understanding how these processes are dynamically regulated is fundamental. Total internal reflection fluorescence microscopy (TIRFM) is ideally suited to study these events. It is based on the use of fluorescent labeled proteins associated to a microscope set-up that allows selective excitation of molecules at the plasma membrane. Indeed, fluorescent molecules alone provide information about the expression and localization of proteins and other molecules, but the temporal and spatial resolution is confounded by signal from outside the area of interest. TIRFM overcomes this limitation by using an evanescent wave generated by the reflection of a laser beam; this wave is relatively low powered and it is able to illuminate just a narrow (<100 nm) strip at the surface of a cell, thereby excluding the signals arising from structures in the cell interior (Figure 1).[1]

As a result, spatial and temporal resolutions are increased, thus making it possible to measure dynamic events occurring at or immediately below the plasma membrane such as exocytosis and endocytosis, single molecule interactions, and ionic changes. This technique allows not only qualitative analysis, but also a quantitative measure of these events, by evaluating variation in fluorescence intensity during time-lapse recording. Analysis of these processes may open novel perspectives in the study of cell signaling, membrane trafficking and cytoskeleton remodeling.

In our laboratory, we have taken advantage of TIRFM for evaluating the glutamatergic signaling in the nervous system and in peripheral organs, in particular we have investigated the following.

First, dynamic modulation of glutamate transporter density at the plasma membrane. The excitatory amino acid carrier 1 (EAAC1) is a plasma membrane high affinity glutamate transporter expressed in the nervous system and in absorptive epithelia. EAAC1 activity can be rapidly regulated by its redistribution between intracellular compartments and the plasma membrane, a process controlled by protein-protein interactions and extracellular signals. We analyzed the molecular mechanisms of this modulation in two different physiological contexts: in epithelial cells, where EAAC1 is important for absorption of dicarboxylic amino acids, and in Schwann cells, where it may participate in cell myelination and proliferation by regulating the level of extracellular glutamate or by providing Schwann cells with glutamate.

In epithelial cells, we investigated the molecular mechanisms that control the surface density of EAAC1. We detected in its cytoplasmic C-tail a consensus sequence for interaction with class I PDZ proteins and a tyrosine-based internalization signal ($-^{503}$YVNG^{506}). To understand their role in transporter trafficking, we generated green fluorescent protein (GFP)-tagged transporters-lacking the PDZ target motif (ΔTSQF) or carrying the Y503A substitution. We expressed them in the Madin Darby Canine Kidney epithelial cell line, and we monitored their residence on the plasma membrane by time-lapse TIRF imaging. In these experiments, if the GFP transporter is internalized, the fluorescence signal recorded by TIRFM should progressively decrease. Quantification of the fluorescence changes in the different mutants indicated that the PDZ target sequence controls the transporter resident time at the plasma membrane and that Y503 is involved in the constitutive endocytosis of EAAC1 (Figure 2).[2]

In Schwann cells, we investigated the mechanisms of action of allopregnanolone (ALLO), a steroid with neuroprotective effects, synthesized by Schwann cells. We found that incubation with ALLO rapidly increases the activity of the glutamate transporter EAAC1, with a mechanism that involves protein trafficking to the plasma membrane. We investigated this phenomenon by time lapse TIRFM imaging in primary cultures of Schwann cells transfected with EGFP-EAAC1. By alternatively blocking the exo- or endocytic pathways, we found that ALLO promotes the surface delivery of EAAC1 and increases its plasma membrane residence time by tethering it to the submembrane cytoskeleton. This recruitment is important to control Schwann cell proliferation.[3]

Second, vesicle dynamics. Vesicle exocytosis is a common mechanism to control neurotransmitter and hormone release in different bio-

Correspondence: Eliana Di Cairano, Department of Pharmacological and Biomolecular Sciences, University of Milan, via Trentacoste 2, 20134 Milan, Italy.
E-mail: eliana.dicairano@unimi.it

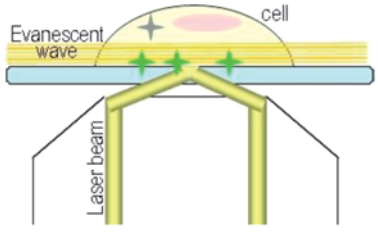

Figure 1. Schematic representation of TIRF microscopy. When the laser beam is reflected by the glass slide, it generates an evanescent wave that diffuses in the specimen with the same wavelength but decades in a short distance, thereby illuminating only fluorescent molecules at or immediately below the plasma membrane.

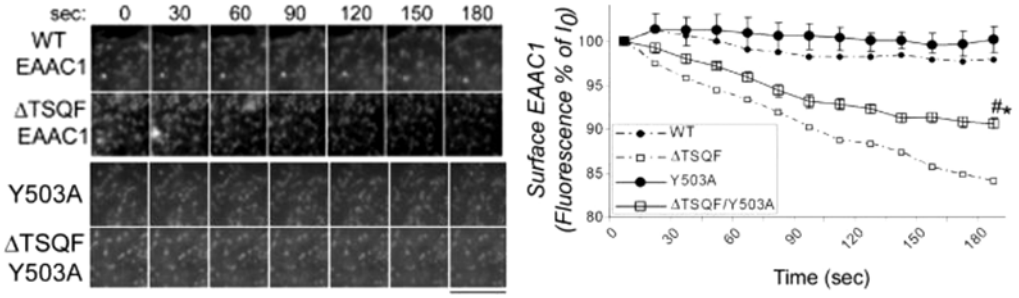

Figure 2. Representative TIRFM image sequences showing the membrane resident time of the indicated transporters (left), together with the averaged fluorescence intensity curves (right).

logical systems. To investigate the molecular mechanisms of vesicle exocytosis modulation, we labeled mice cortical synaptosomal membranes with the fluorescent organic dye FM1-43. Under TIRFM, we monitored the effect of corticosterone treatment on dynamics of vesicles docking and fusion and we found that the glucocorticoid promotes the docking of vesicles to the synaptic plasma membrane.[4]

In addition to conventional fluorescent dyes, genetically engineered fluorescent proteins such as vesicles-resident proteins, cargo molecules (neurotransmitter and hormones) are increasingly being used to measure membrane trafficking and to monitor cell signaling.[5] We are currently setting up the experiments to measure the dynamics of hormone release in endocrine cells of the pancreas.

References

1. Axelrod D. Total internal reflection fluorescence microscopy in cell biology. Traffic 2001;2:764-74.
2. D'Amico A, Soragna A, Di Cairano E, et al. The surface density of the glutamate transporter EAAC1 is controlled by interactions with PDZK1 and AP2 adaptor complexes. Traffic 2010;11:1455-70.
3. Perego C, Di Cairano ES, Ballabio M, Magnaghi V. Neurosteroid allopregnanolone regulates EAAC1- mediated glutamate uptake and triggers actin changes in Schwann cells. J Cell Physiol 2012;227:1740-51.
4. Treccani G, Musazzi L, Milanese M, et al. Stress at the synapse: the synaptic action of acute behavioural stress and the protective effect of psychiatric drugs. Eur J Neuropsy 2011;21:22-3.
5. Ohara-Imaizumi M, Nishiwaki C, Kikuta T, et al. TIRF imaging of docking and fusion of single insulin granule motion in primary rat pancreatic beta-cells: differentm behaviour of granule motion between normal and Goto-Kakizaki diabetic rat beta-cells. Biochem J 2004;381:13-8.

Influence of flavonoids on the transmembrane electron transport: study *ex-vivo*

D. Di Majo,[1] M. La Guardia,[2] M. Crescimanno,[1] C. Flandina,[1] G. Leto,[1] M. Giammanco[1]

[1]Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo; [2]Division of Cell Biology, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

Introduction

The oxidative stress results from a change in the physiological balance between oxidant and antioxidant species. This condition induces an chemical change in the redox state of cells. Ever growing evidence has demonstrated that the trans- Plasma Membrane Electron Transport (t-PMET) occurs in all types of organisms, including bacteria, yeast plants and animals.[1] The function of t-PMET is to cooperate with intracellular redox pairs, like piruvate and lactate, to maintain the cytoplasmatic NAD^+/NADH balance. The t-PMET is correlated with the modulation of internal pH and redox homeostasis, as it is able to activate proton release.[1] Thus, t-PMET causes external and internal pH modification, as well as development of an inside negative membrane potential. These changes are responsible of cellular functions alterations, including cell-volume sensing and regulation; mitogenic/apoptogenic signalling and transport across membranes[1] The malfunction of the tPMET is relates to some diseases such as cancer, cardiovascular diseases, aging, obesity, neurodegenerative diseases, pulmonary fibrosis, asthma.[1] The activity of t-PMET is critical to redox homeostasis in blood. In hypoxic condition the activation of t-PMET may serve to compensate the impaired pentose phosphate pathway, thus ensuring a functional reducing capacity; in this conditions t-PMET may use ascorbic acid or polyphenols as electron donator, since NADH derived from enhanced glycolysis is preferentially utilized by meta-hemoglobin reductase.[2] The aim of this work was evaluate the erythrocytes redox status in a group of health volunteers and then to study whether some of flavonoids, enclosed in sub-class of flavonols (Quercetin and Kaempferol), are able to modify the erythrocytes redox homeostasis. Our attention has been focused on red blood cells (RBC) because a close link between t-PMET and metabolic status of erythrocytes has been reported.[3] The RBCs act as antioxidants to themselves and, in addition, their mobility carries their antioxidant capabilities to all the plasma accessible parts of the body.

Correspondence: Danila Di Majo, Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, viale delle Scienze, Ed. 16, 90127 Palermo, Italy.
E-mail: danila.dimajo@unipa.it

Journal of Biological Research 2015; 88:5161

Methods

The subjects that participating at this work attested no supplement intake or some other substance that could interfere with our tests. The protocol of the study was in conformity with the guidelines of the Institutional Ethical Committee. Human venous blood from twenty healthy volunteers of both sexes between the ages of 25-50 years were obtained by venipuncture in heparin after an over-night fast and centrifuged. The antioxidant capacity of plasma was analyzed by crocin bleaching assay[4] and FRAP.[5] On the other hand, the reducing activity in erythrocytes that represents the body redox state of the last 120 days was evaluated by FRAP method.[5] After centrifugation at 3000 rpm for 10 minutes at 4°C, plasma was separate from red blood cells. The resultant plasma was transferred to microcentrifuge tubes and used at least in part for ferric-reducing activity power (FRAP) and for Crocin Bleaching Assay (CBA) and the others aliquots was stored -80°C before to use for further analysis. Red blood cells, after removal of buffy coat and upper 15% of the packed red blood cells, were washed two times with cold PBS according to Fiorani et al. method (2005). A stock solution (20mM) of each flavonoid was prepared in dimethyl sulfoxide and then diluted 1:2 with PBS. Packed RBC (10%v/v) were incubated in PBS containing 5mM glucose at 37°C for 10. minutes with a 50 μM concentration of each flavonoids. After this time the suspensions was centrifuged, the RBC were washed and then analyzed. The resulting supernatant fractions were assayed for their FOC content using 1,10-phenanthroline as an indicator and measuring absorption at 510 nm. The results are expressed in mol ferrocyanide/mL RBC/30 min, through Lamber-Beer law, ε=10500 M-1cm-1. The percentage of hemolysis was evaluated in the same sample by measuring the haemoglobin contents. The Drabking test, purchased from Sigma-Aldrich, has been used to evaluate the haemoglobin contents. The extent of lysis was not different than the controls and never higher than 0.5%.

Results

Table 1 shows the values of plasma and erythrocyte antioxidant status of health volunteers. All the values confirm the data of literature[6,7] about the antioxidant status of human being in physiological condition. All compounds were taken up by the erythrocytes and displaying significant FIC-reducing activity. Figure 1 reports the values of reducing capability of the control (erythrocytes without flavonoids) towards

Table 1. Plasma and erythrocyte antioxidant status and membrane redox system values of the volunteers.

Plasma Antioxidant activity by CBA (Ka/Kc)	Erythrocytes Reducing activity by FRAP (μmol/L)	Erythrocytes reducing activity (μmol/L)	tPMET (μmol ferrocyanide/mL PBRC/30min)
20.69±6.35	576±25	1520±258	268±0.16

Data are expressed as means±standard deviation.

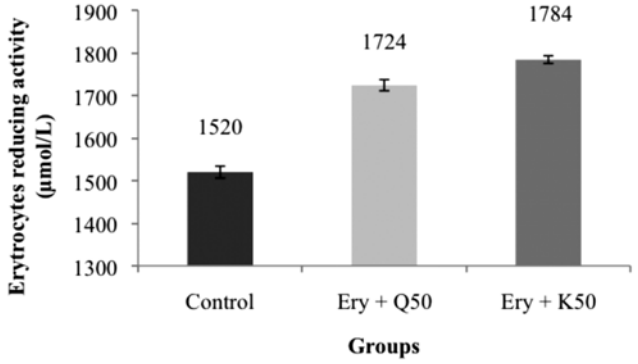

Figure 1. Effect of the Quercetin and Kaempferol at the concentration of 50μM on the reducing activity of erythrocytes. Data are expressed as mean±standard deviation. Statistical differences were analyzed with the Student's unpaired t-test. Control *vs* Q50 (P=0.02); control *vs* K50 (P=0.02); Q50 *vs* K50 not significant.

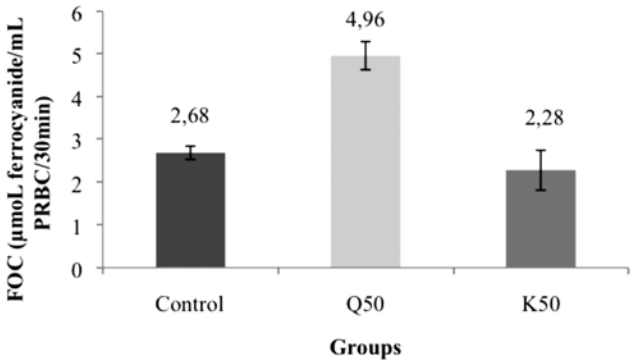

Figure 2. Effect of the Quercetin and Kaempferol at the concentration of 50μM on the t-PMET system of erythrocytes. The date are expressed as media±SD. Statistical differences were analyzed with the Student's unpaired t-test. Control *vs* Q50 (P=0.02); control *vs* K50 not significant.

the erythrocytes and of the erythrocytes incubated with quercetin and kaempferol at the concentration of 50 μM. Both the analysed compounds (Quercetin and Kaempferol) are able to increase the reducing activity of the erythrocytes of 15% and 13% respectively respect to the value recorded for the control. On the other hand, only the quercetin was able to increment the activity of the tPMET system; not any significant difference has been recorded between the Kaempferol and the control group (Figure 2).

Conclusions

This study shows that the flavonoids are able to form stable complexes with the erythrocytes and to influence the intracellular redox homeostasis. Therefore, it could affirm that the polyphenols are able to increase the defence of erythrocytes against ROS. This work underlines that the RBC plays a pivotal role in the distribution and bioavailability of circulating polyphenols which contribute to the defence against injury induced by ROS in various clinical disorders.

References

1. Del Principe D, Avigliano L, Savini I, Catani MV. Trans-plasma membrane electron transport in mammals: functional significance in health and disease. Antioxid. Redox Signal 2011;11:2289-318.
2. Kennett EC, Kuchel PW. Plasma membrane oxidoreductase: effects on erythrocyte metabolism and redox homeostasis. Antioxid Redox Signal 2006;8:1241-7.
3. Rogers SC, Said A, Corcuera DD, et al. Hypoxia limits antioxidant capacity in red blood cells by altering glycolytic pathway dominance. FASEB J 2009;23:3159-70.
4. Tubaro F, Ghiselli A, Rapuzzi P, et al. Analysis of plasma antioxidant capacity by competition kinetics. Free Rad Biol Med 1998;24:1228-34.
5. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem 1996;239:70-6.
6. Fiorani M, Accorsi A. Dietary flavonoids as intracellular substrates for erythrocyte trans-plasma membrane oxidoreductase activity. Br J Nutr 2005;94:338-45.
7. Kampa M, Nistikaki A, Tsaousis V, et al. A new automated method for the determination of the Total Antioxidant Capacity (TAC) of human plasma, based on the crocin bleaching assay. BMC Clin Pathol 2002;2:3.

Influence of quercetin and luteolin on the activity of the catalase: study *ex vivo* about erythrocytes in smokers and non-smokers

D. Di Majo,[1] M. La Guardia,[2] E. Di Sclafani,[1] C. Flandina,[1] M. Crescimanno,[1] G. Leto,[1] M. Giammanco[1]

[1]Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo; [2]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

Introduction

Oxygen free radicals are highly reactive species that can cause cell damage including lipid peroxidation, enzyme inactivation and DNA damage. Inactivation and removal of highly reactive species depend by the antioxidant defence systems. The catalase (CAT) belongs to the primary antioxidant defence system of the cell which catalyses the decomposition of hydrogen peroxide to water and molecular oxygen. Flavonoids are a group of polyphenolic compounds with different chemical structure and properties. They are widely distributed in fruits, vegetables, nuts, seeds and beverages such as wine and tea. The major flavonoide classes include flavonols, flavone, flavonones, flavanols, anthocyanidins and isoflavones. These compounds may scavenge free radical species and other oxidants. In previous studies, we have observed that some polyphenols are able to cross the erythrocyte's membrane and this process is influenced by the plasmatic albumin; in fact quercetin intracellular concentrations is albumin dose depending. The influence of flavonoids on catalase activity has been reported in some papers but the results are contradictory. Some authors have found an increase of catalase activity in cell in the presence of flavonoids.[1,2] Others have observed any effect or even a decrease of catalase activity.[3] The present work is based on a study of Krych's,[4] in which the influence of flavonoids on catalase in model system has been evaluated. Aim of this work was to study the role of red body cells in the antioxidant balance. The primary goal was to evaluate the antioxidant status of no-smokers and ever-smokers healthy subjects by the determination of the plasma antioxidant capacity and of the catalase activity of erythrocyte and then to evaluate if flavonoids (quercetin and luteolin) are able to modify the enzyme activity.

Correspondence: Danila Di Majo, Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, viale delle Scienze, Ed. 16, 90127 Palermo, Italy.
E-mail: danila.dimajo@unipa.it

Journal of Biological Research 2015; 88:5161

Methods

This is a pilot study. Nine healthy subjects, aged 24-55 years, of which six females (3 no-smokers and 3 ever smokers) and 3 males (2 smokers and 1 no-smokers) were recruited. None of the subjects had any pathologies at the time of sampling. We assayed the CAT activity in erythrocytes isolated from whole blood of the subjects by the colorimetric assay and the plasma antioxidant capacity using the spectrophotometric method known *crocin bleaching assay*. The catalase activity was performed in human erythrocytes (control) and after the incubation of them with the flavonoids (quercetin and luteolin). Human venous blood (in heparin) from healthy volunteers was obtained by venepuncture. The blood was centrifuged and then plasma, buffy coat and upper 15% of the packed erythrocytes were removed. The isolated erythrocytes isolated were washed twice with cold PBS and then re-suspended and incubated with flavonoids according Fiorani[5] method. The catalase activity was analysed by a catalase assay kit purchased from Sigma-Aldrich. All results are presented as mean±SD or mean±SEM. Correlation statistics between variables were assessed by calculating the Pearson coefficient. Differences in means between groups were analyzed by the unpaired t-test. Differences were considered statistically significant at $P<0.05$. Multiple regression analysis was used to investigate the influence of different variables on the enzyme activities.

Results

The flavonoids were efficiently taken up by human erythrocytes in dose-depending manner. There was no significant difference in the percent accumulation of both molecules (quercetin and luteolin) inside the erythrocytes when incubated at the same concentration of 50μM. In physiologic condition the catalase activity varies from 28.6 mU/g protein to 40.6 mU/g protein. Data have shown that CAT activity of erythrocytes was significantly lower in ever smokers than in no-smokers (Figure 1). It was also found that Quercetin at the concentration of 100μM is able to increase the catalase levels in ever-smokers up to the normal values observed in no smokers (Figure 2). The study on the luteolin has not produced the same effects. In fact, Luteolin is able to reduce the CAT levels in no-smokers subject according with the data from Krych.[4] The different actions of compounds on catalase can been explained as consequence of flavonoid interaction with enzymatic protein. The inhibiting action of the luteolin can be a consequence of a conformational change which occurs upon the flavonoid binding to catalase. This interaction changes the geometry of the substrate channel and thus inhibits the reaction of H2O2 with the heme center.

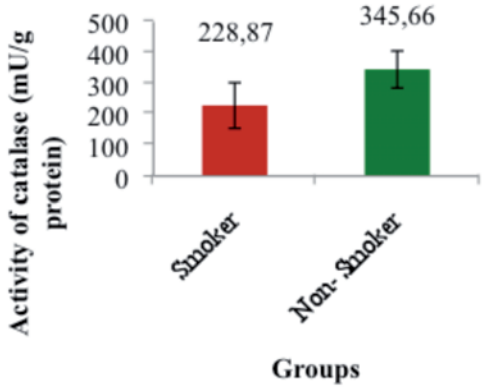

Figure 1. Catalase activity of erythocytes in non-smokers and ever-smokers. Data are expressed as mean±SEM (unpaired t-test; P=0.007).

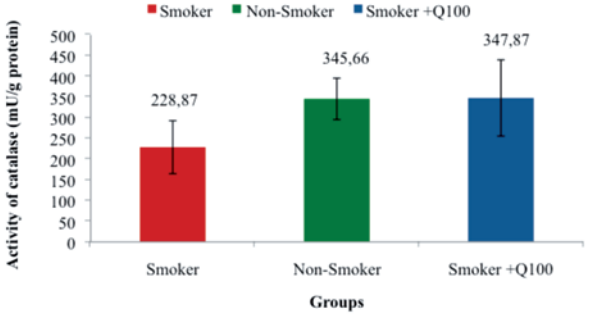

Figure 2. Effect of quercetin (100μM) on the catalase activity in ever-smokers. Data are expressed as mean±SEM. Non-smokers *vs* smokers +Q100 (P=0.09).

Plasma antioxidant capacity was lower in no smokers than in smokers. An inverse correlation has been found between age and plasma antioxidant capacity.

Conclusions

From the results of this study affirms that an oxidative stress condition is present in ever-smokers respect to non-smokers, but the quercetin is able to restore the erythrocyte oxidative stress condition of ever-smokers back to the normality. Further studies are necessary in the future to better investigate the role of luteolin on the catalase activity of human erythrocytes. Wu et al. have warned that microenvironment can shift erythrocytes from friendly to a harmful behaviour. The release of iron-hemoglobin following RBC hemolysis can cause excess accumulation of free iron to catalyze the generation of the highly toxic hydroxyl, peroxyl and alkoxyl radicals. Therefore by virtue of their antioxidant and chelating properties for divalent metals both free and polyphenols bound to RBC might also act to neutralize the toxic effects of ROS.

References

1. Elavarasan J, Velusamy P, Ganesan T, et al. Hesperidin-mediated expression of Nrf2 and upregulation of antioxidant status in senescent rat heart. J Pharm Pharmacol 2012;64:1472-82.
2. Kampkötter A, Chovolou Y, Kulawik A, et al. Isoflavone daidzein possesses no antioxidant activities in cell-free assays but induces the antioxidant enzyme catalase. Nutr Res 2008;28:620-8.
3. Valdameri G, Trombetta-Lima M, Worfel PR, et al. Involvement of catalase in the apoptotic mechanism induced by apigenin in HepG2 human hepatoma cells. Chem-Biol Interact 2011;193:180-9.
4. Krych J, Gebicka L. Catalase is inhited by flavonoide. Int J Biol Macromol 2013;58:148-53.
5. Fiorani M, Accorsi A. Dietary flavonoids as intracellular substrates fora n erytrocyte trans- plasma membrane oxidoreductase activity. Brit J Nutr 2005;94:338-45.

The approach of microRNA expression analysis in the detection of autologous blood transfusion in doping control

F. Donati,[1] F. Boccia,[1] A. Stampella,[1] X. de la Torre,[1] F. Botrè[1,2]

[1]Dope Testing Laboratory, Italian Sport Medicine Federation, Rome; [2]Management Department, University La Sapienza, Rome, Italy

Introduction

Blood transfusion (BT) as blood doping practice is banned by the World Anti-Doping Agency (WADA) and can be abused by cheating athletes to increase the rate of oxygen transport to tissues with the aim to improve sport performance. At present, a method for the detection of Homologous Blood Transfusion (HBT) has been implemented by the WADA accredited antidoping laboratories worldwide, while no internationally recognized method has been finalized so far for the direct detection of autologous blood transfusions (ABT), which can at present be only detected indirectly by targeting longitudinal profiling of key hematological parameters. In this perspective, several researches approaching different fields are underway to find reliable biomarkers to be suitable in the development of a method to directly detect autologous blood transfusion. In this work we have experimented the possible role of microRNA (miRNAs) as biomarkers related to the execution of an autologous blood transfusion practice. MiRNAs are a class of 18-24 nucleotides long non coding RNAs acting as post-trancriptional modulators of mammalian gene expression. Normally they are involved in the regulation of many physiological processes (such as erythropoiesis), and recent evidences shown that they have a role as useful biomarkers in certain diseases such as cancer and heart malignancies. This study aims to experiment their possible role as biomarkers of blood doping.

Materials and Methods

A total of eight miRNAs (mi923, mi150, mi144, mi96, mi196a, mi30b, mi197, mi451) have been extracted and quantified from six whole blood samples from healthy athletes at three different times (T=0 within 24h from sample taking, T=1 after 15 days, T=2 after 30 days). Another blood sample was withdrawn fresh from a donor, then stored as erythrocyte concentrated and, after 30 days, used to get an *ex-vivo* autologous blood transfusion with new fresh blood from the same donor. All miRNAs were extracted with a specific kit (miRneasy, Qiagen), then quantified with a specific Chip Electrophoresis System (Bioanalyzer 2100, Agilent), then retro-transcribed to cDNA and analyzed to quantitative PCR (qPCR) using PCR7500 Fast system (Applied biosystems). Analysis of expression was made with the technique of *relative quantification* that was choosen as the more suitable for the aims of this experiments. In data analysis, a key step was the identification of the most appropriate housekeeping gene who resulted mi150 as its variability was found to be the most stable among all miRNAs studied. The study of the variation of the expression was evaluated estimating the relative quantities (RQ) of the miRNAs expressed by the samples compared to a calibration sample used as reference.

Results and Discussion

We observed a gradual tendency of the miRNAs analyzed to increase their expression in samples at T=2 compared to T=0 where mi144 and mi923 shown the most consistent differences however with great variability among the samples. A marked differences has been observed for the erythrocytes concentrate sample (analyzed after 30 days of storage) where expression levels of mi923, mi30b, mi197, mi96 and mi451 resulted higher compared to the fresh samples at the time of the withdrawal. Moreover the most important result relies on the observation that the expression of some miRNAs (such as mi197, mi30b, mi451, mi96 and mi923) is very high in the erythrocytes concentrated sample and it is detectable also in the ex- vivo transfused sample with levels higher compared to the fresh non-transfused sample (even though lower compared to the concentrated erythrocytes sample because of the post-transfusion dilution effect). Moreover, two miRNAs (mi144 and mi923) show the most significant increasing in the transfused sample compared to the fresh one. As result, it is very important to note that, from the data obtained, the possibility to use miRNAs expression both as biomarkers of storage and biomarkers of effect in blood doping detetction emerges. Results we achieved in this work seem to be significant for several reasons. Firstly, at methodological level, the development of a specific protocol for the extraction and the quantitation of miRNA and a proper strategy in data analysis (including a correct strategy in the choice of the most suitable *housekeeping gene*) have allowed for the first time our laboratory to experiment and apply molecular biology techniques in the field of doping control. More in detail, an accurate and sensitive quantification of the extracted miRNAs represented a first key step to sub-

Correspondence: Francesco Donati, Dope Testing Laboratory, Italian Sport Medicine Federation, largo Giulio Onesti 1, 00197 Rome, Italy.
E-mail: donati.francesco@gmail.com

Journal of Biological Research 2015; 88:5161

sequently achieve accurate and reproducibile genetic expression data by quantitative real time PCR.

Conclusions

Finally, the methodological approach here followed and results obtained in this work can be considered as preparatory to the opening of new research perspectives in the field of doping control. The next step we aim for the future is first of all to extend both the panel of miRNAs to quantitate and the number of samples to be analyzed also considering the important issue of ethnical diversity between individuals. Moreover, the strategies and studies we intend to apply in the near future also include the extention of this approach to different hematological matrixes considering for example circulating miRNAs in serum and plasma and, in a further step, considering miRNAs extracted from urine samples also to the final scope to experiment and apply miRNAs expression data as biomarkers for the detection of other banned doping practices and drugs as for example the abuse of synthetic erythropoietins, insulin and analogs, growth hormone and related growth factors.

Identificazione tassonomica di *Aotus* (Platyrrhinae) mediante la citogenetica

F. Dumas,[1] L. Sineo,[1] T. Ishida[2]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy; [2]Graduate School of Science, University of Tokyo, Japan

Le scimmie del genere *Aotus* (Platyrrhine, Cebidae) presentano caratteristiche peculiari essendo notturne e monogame. Hanno un'ampia distribuzione geografica che si estende dallo stretto di Panama al nord dell'Argentina; questo determina una notevole complessità nella distribuzione e di conseguenza nell'interpretazione sistematica e filogenetica. Lo studio del genere *Aotus* mediante l'analisi di caratteri morfologici e genetici ha prodotto una tassonomia controversa. Inizialmente era riconosciuta solo la specie *Aotus trivirgatus*, successivamente in base alla colorazione del pelo del collo, alla diversa suscettibilità alla malaria e ai dati citogenetici sono state identificate fino a nove specie, oltre a due *sibling species*, per un totale di 11.[1,2] Il numero diploide di cromosomi in *Aotus* varia da 46 a 56; nel cariotipo sono presenti molti polimorfismi dovuti all'ibridazione che si verifica in specie simpatiche; in alcune specie è presente una traslocazione Y-autosoma. Le undici specie sono state suddivise in due gruppi monofiletici: il gruppo *grey-black neck* distribuito a nord e il gruppo *red neck* distribuito a sud del Rio delle Amazzoni. Le specie del gruppo *red neck* sono omogenee da un punto di vista del cariotipo con un numero diplolide 2n=49 (maschio)/50 (femmina) e una traslocazione Y- autosoma. Le specie del gruppo *grey-black neck* presentano numero diploide di cromosomi variabile, con il più basso 2n=46 in *A. vociferans* e il più alto 2n=56 in *A. lemurinus*. Solo due specie (*Aotus nancymae* e *Aotus lemurinus*) sono state analizzate mediante la citogenetica molecolare (*painting* cromosomico). Quest'analisi ha permesso di dimostrare che le specie del genere *Aotus* posseggono un cariotipo piuttosto derivato se confrontato con quello ipotetico ancestrale delle Platyrrhinae, da cui si è originato attraverso fusioni, fissioni, traslocazioni ed inversioni.[3-5]

L'identificazione tassonomica di taxa di *Aotus* mediante l'analisi cromosomica rappresenta, in cattività, il prerequisito per programmi di *breeding* in quanto la ricostruzione del cariotipo bandeggiato è l'unico approccio per identificare la maggior parte delle specie del genere *Aotus*. Mediante le tecniche citogenetiche vengono identificati individui compatibili da un punto di vista cromosomico che possono essere incrociati tra loro al fine di evitare ibridazioni interspecie[6] e favorire la conservazione delle diverse specie.

Figura 1. Cariotipo ricostruito mediante bandeggio g di un individuo di *Aotus* l. *griseimembra* (2n=54) proveniente dal centro Giapponese; un maschio con un grande cromosoma submetacentrico risultato di una fissione (prima riga, primo cromosoma) e due cromosomi non omologhi (seconda riga ultimi due cromosomi); ricostruzione in accordo con Ma e colleghi.[2]

L'obiettivo del presente lavoro riguarda l'identificazione mediante bandeggio cromosomico di individui di una colonia di *Aotus,* al fine di avviare un programma di conservazione delle specie mediante *breeding.* Il gruppo di scimmie, originario della Bolivia, è presente in Giappone dal 1977 presso The Primate Research Institute di Tokyo; tra gli individui della colonia si è inavvertitamente verificata la produzione di ibridi prima che fossero riconosciute le diverse specie del genere. Si riportano dati preliminari sull'identificazione di individui idonei da incrociare, in particolare il cariotipo bandeggiato di un maschio di *A.l griseimbra* (2n=54) (Figura 1). Inoltre si sono revisionati dati citogenetici presenti in letteratura su *Aotus* al fine di sottolineare l'importanza della citogenetica classica e molecolare negli studi filogenetici e in quelli riguardanti la conservazione delle specie (Tabella 1).

Correspondence: Francesca Dumas, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, via Archirafi 18, 90127 Palermo, Italy.
E-mail: francesca.dumas@unipa.it

Journal of Biological Research 2015; 88:5161

Bibliografia

1. Hershkovitz P. Two new species of night monkeys, genus Aotus (Cebidae, Platyrrhini): a preliminary report on Aotus taxonomy. Am J Primatol 1983;4:209-43.
2. Ma NS, Jones TC, Miller AC, et al. Chromosome polymorphism and banding patterns in the owl monkey (Aotus). Laboratory Animal Science 1976;26:1022-36.
3. Stanyon R, Bigoni F, Slaby T, et al. Multi-directional chromosome painting maps homologies between species belonging to three genera of New World monkeys and humans. Chromosoma 2004;113:305-15.

Tabella 1. Elenco delle specie analizzate ad oggi mediante la citogenetica classica e molecolare con il relativo riferimento bibliografico.

NAME	2N=	METHODS	REFERENCES	DATE
Aotus (from Colombia)		R,Q,G-NOR-banding	Torres et al.,	1998
Aotus trivirgatus	56	G- banding	Chiarelli and Stanyon	1985
Aotus trivirgatus-from Peru	49, 50 female	G , C banding	Pieczarka and Nagamachi	1988
Aotus		Review.	Galbreath	1983
Aotus trivirgatus form Peru	46,47,48	G- banding	ShuiFong Ma et al.,	1985
Aotus		chromosome evolution	shui Fong and Ma	1981
Aotus (from Northern Argentina)	50 female, 49 male	C, G- banding	Mudry and Colillas	1984
Aotus		Chromosome Nomenclature	Reumer, De Boer	1980
Aotus (from Bolivia)	50 female, 49 male	C, Q, G- banding,	Ma et al.,	1976
Aotus (from Rondonia, brazil)	48	G, C, NOR- banding	Pieczarka et al.,	1993
Aotus nancymae, *A. vociferans*	54 46	G, C, NOR- banding	Pieczarka et al.,	1992
Aotus			de Boer	1974
Aotus nancymae	54	FISH	Stanyon et al.,	2004
Aotus nancymai *Aotus sp*	54 50	FISH	Ruiz Herrera et al.,	2005
Aotua jorgehernandezi	50	G- banding G- banding	Defler TR, Bueno Torres et al.,	2007 1998
Aotus lemurinus	54	FISH	Stanyon et al.,	2011

4. Ruiz-Herrera A, Garci AF, Aguilera M, et al. Comparative chromosome painting in aotus reveals a highly derived evolution. Am J Primatol 2005;65:73-85.
5. Stanyon R, Garofalo F, Steinberg ER, et al. Chromosome painting in two genera of South American monkeys: species identification, conservation, and management. Cytogenet Genome Res 2011;134:40-50.
6. Kumamoto Arlene TA, Houck ML. Cytogenetic identification of a hybrid owl monkey, Aotus nancymaae × Aotus lemurinus griseimembra. J Zoo Wildl Med 2001;32:130-3.

Feeding and cortisol alter branchial Na^+/K^+ ATPase activity and growth performance differently in common carp *Cyprinus carpio*

A. Fazio,[1] L. Hon Jung,[2,3] G. De Boeck,[2] C. Faggio[1]

[1]Department of Biological and Environmental Sciences, University of Messina, Italy; [2]Systemic Physiological and Ecotoxicological Research Unit, Department of Biology, University of Antwerp, Belgium; [3]Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Malaysia

Feeding is the key of growth and survival, granting sufficient nutrients in all living life. Feeding is under endocrine monitoring, and the role of cortisol on feeding modulation is poorly understood: whether the growth-suppressing is due to a cortisol-mediated decrease in food intake is not clear.[1] On the other hand, cortisol is the main circulating corticosteroid in teleost, plays a significant role in monitoring homeostasis balancing in freshwater fish. However, most of the researches were focused on freshwater salmonid and not much is known about cyprinid. Therefore, the aim of this study was to investigate the effect of cortisol on the growth performance, branchial Na^+/K^+-ATPase (NKA) activity and plasma Na^+ levels in common carp *Cyprinus carpio*.

Carp was fed at low and high ratio (0.5% and 3.0% body weight (BW)) for 6 weeks. Carp was injected intraperitoneally with coconut oil implant (sham), or cortisol in coconut oil implant (cortisol–250 mg cortisol per kg fish) while control fish received no injection, and was monitored at 12, 24, 72 and 168 hours after injection (h-PI). Cortisol decreased growth rate compared to sham and control groups, in the high-fed group (Table 1). High feeding significantly increased gill NKA activity in sham and cortisol implanted fish at 24h-PI and in all groups at 72h-PI compared to low-fed fish (Figure 1). Cortisol injection did not affect plasma Na^+ level, although a decreasing trend was observed in the high-fed group at 24h-PI. Furthermore, plasma Na^+ levels were increased at 168h-PI in the high-fed group after cortisol injection compared to the low-fed group. Plasma Na^+ levels were enhanced in the high-fed group due to dietary intake (Table 1).

Cortisol increased branchial NKA activity: the upregulation of this activity further confirms the role of cortisol in modulating ionoregulation capacity in freshwater fish.

The lower growth rate observed in cortisol treated-fish fed to high ratio reflected a high living cost,[2-4] despite the high feeding ratio ensures an appropriate nutrient supply.

As a conclusion, cortisol impaired weight gain in common carp but reallocated energy to compensate increasing NKA activity for basal homeostasis needs.

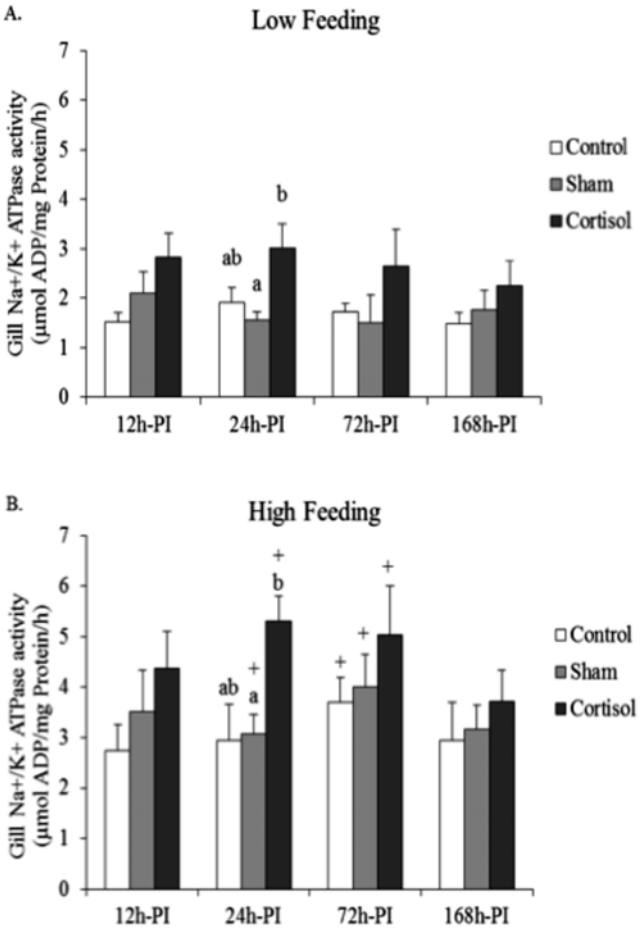

Figure 1. Gill Na+/K+ ATPase activity of common carp fed at low (1A) and high (1B) ratio. All values were presented as mean±SEM, *n*=8. Significant level was set at P<0.05. A plus (+) indicates significant differences between feeding regimes.

Correspondence: Angela Fazio, Department of Biological and Environmental Sciences, University of Messina, viale Ferdinando Stagno D'Alcontres 31, 98166 Messina, Italy.
E-mail: angi170@alice.it

Table 1. Growth rate (%) and Plasma Na^+ (mmol/L) of common carp fed at different ratio without (control) and with implantation (sham and cortisol) over 168h-PI. All values were presented as mean±SEM, *n*=8. Significant level was set at P<0.05. A plus (+) indicates significant differences between feeding regimes.

		Low feeding			High feeding		
		Control	Sham	Cortisol	Control	Sham	Cortisol
Growth rate (%)	12 h-PI	3.2±0.9	0.7±0.4	1.0±0.4	9.6±0.4+	2.2±0.8	1.5±0.9
	24 h-PI	4.2±0.6	1.6±1.0	1.8±0.5	6.2±0.7	2.2±0.7	2.4±0.8
	72 h-PI	2.3±1.0	1.1±0.3	0.8±0.5	5.3±0.4+	2.0±0.4	2.2±0.6
	168 h-PI	2.2±0.7	0.8±0.1	0.8±0.1	7.0±1.0+	4.1±0.9+	2.5±0.7
Plasma Na^+ (mmol/L)	12 h-PI	119±4.6	113±4.9	107±2.9	131±5.8	126±5.3	118±4.6
	24 h-PI	122±1.5	114±5.5	110±2.7	136±1.8+	123±5.1	115±7.2
	72 h-PI	121±2.2	116±4.3	111±3.7	133±4.1+	127±6.9	119±6.3
	168 h-PI	118±7.0	113±6.1	109±2.9	130±1.8	125±4.6	124±4.9+

References

1. Bernier NJ, Peter RE. Appetite-suppressing effects of urotensin I and corticotropin-releasing hormone in goldfish (Carassius auratus). Neuroendocrinology 2001;73:248-60.
2. Bernier NJ, Bedard N, Peter RE. Effects of cortisol on food intake, growth, and forebrain neuropeptide Y and corticotropin-releasing factor gene expression in goldfish. Gen Comp Endocrinol 2004; 135:230-40.
3. De Boeck G, Alsop D, Wood CM. Cortisol effects on aerobic and anaerobic metabolism, nitrogen excretion, and whole-body composition in juvenile rainbow trout. Phys Biochem Zool 2001;74:858-68.
4. Gregory TR, Wood CM. The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. Phys Biochem Zool 1999;72:286-95.

Caratteristiche nutrizionali dell'olio di *Moringa oleifera*

E. Finotti,[1] G. Rossoni,[1] I. Garaguso,[1] M. Giammanco,[2] D. Di Majo[2]

[1]CRA-NUT-Food and Nutrition Research Center, Rome; [2]Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, Italy

Introduzione

Tutte le specie di Moringa sono originarie delle regioni Sub-Himalaiane e la più diffusa risulta essere la *Moringa oleifera* v. Lamarck attualmente coltivata in diversi paesi dell'Africa, dell'Asia e Sud-America. Dalla fine degli anni ottanta si è registrato un crescendo di lavori centrati sulla possibile applicazione in campo alimentare, farmaceutico, cosmetico[1] nonché agricolo delle radici, delle foglie, dei semi oppure dell'olio di moringa. Sono apparse una miriade di pubblicazioni che, spesso attingendo dalla tradizione e/o medicina popolare, evocano per i vari derivati di moringa proprietà iperboliche, miracolistiche se non magiche.[2] Di fatto pochi lavori esaminano rigorosamente la chimica dei costituenti e rari sono gli studi clinici[2] che ne documentano l'efficacia nutrizionale in modo puntuale.[2-4] D'altra parte, nel sahel senegalese, zona da cui provengono le foglie e i semi oggetto di questo studio, l'albero di moringa è considerato una risorsa polivalente. Il legno è impiegato in falegnameria o come legna per ardere, le foglie fresche vengono utilizzate per integrare l'alimentazione se seccate e ridotte in polvere vengono impiegate per *combattere* il diabete. Dell'olio ne fanno un uso culinario nonché cosmetico. Scopo di questo studio preliminare e quello di monitorare e le capacità antiossidanti e il profilo degli acidi grassi introducendo un semplice, ma efficace modo per calcolare la relazione tra saturi ed insaturi, che aiuta a chiarire la notevole resistenza dell'olio di moringa all'ossidazione.

Materiali e Metodi

Il profilo degli acidi grassi è stato effettuato mediante trans metilazione[5] e analizzati in gas-cromatografia con rivelatore di massa. La concentrazione dei polifenoli totali liberi è stata eseguita mediante il Folin-Ciocolteau.[6] La capacitaà antiossidante è stata misurata mediante lo sbiancamento della crocina.[7]

Correspondence: Enrica Finotti, CRA-NUT-Food and Nutrition Research Center, via Ardeatina 546, 00178 Rome, Italy.
E-mail: enrico.finotti@entecra.it

Journal of Biological Research 2015; 88:5161

Risultati e Discussione

Come si può osservare dalla Tabella 1 l'olio di moringa presenta un profilo in acidi grassi molto simile a quello dell'olio di oliva, specialmente nel C18:1, in più nell'olio di moringa è presente in modo cospicuo il C22:0. In Tabella 2 sono riportati i valori dei polifenoli e della capacità antiossidante dell'estratto acqua:metanolo delle foglie di moringa, comparati con quelli di altri prodotti alimentari. I valori per vini e per il the bianco sono riportati in Di Majo e Giammanco.[8] Come è possibile osservare dalla tabella l'estratto alcolico presenta eccellenti valori sia di capacità antiossidante che di concentrazione di polifenoli totali liberi. In questo studio abbiamo voluto introdurre un diverso approccio matematico rispetto ai calcoli che comunemente si fanno per quanto riguarda il rapporto Sat/Ins. A nostro avviso questo tipo di calcolo è fuorviante, in quanto non tiene conto del grado d'insaturazione dei diversi acidi grassi insaturi. Però anche il solo grado d'insaturazione non è esaustivo, perché non considera assolutamente gli acidi grassi saturi, perciò abbiamo introdotto un altro tipo di rapporto e precisamente il rapporto Saturi/Grado d'insaturazione.

Conclusioni

In conclusione, mediante questo nuovo rapporto si evidenzia come l'olio di moringa si discosta nettamente dall'olio extravergine di oliva, cosa non molto evidente con gli altri due indici (Sat/Ins, e Grado d'insaturazione), collocandolo tra l'olio extravergine di oliva e lo strutto. Si è inoltre verificato il buon corredo antiossidante custodito dalle foglie di questa pianta.

Bibliografia

1. Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: a food plant with multiple medicinal uses. Phytother Res 2007;21:17-25.
2. Fahey JW. Moringa oleifera: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Trees Life J 2005;1:5.
3. Abdulkarim SM, Lai OM, Muhammad SKS, et al. Frying quality and stability of high-oleic Moringa oleifera seed oil in comparison with other vegetable oils. Food Chem 2007;105:1382-9.
4. Tsaknis J, Lalas S. Stability during frying of Moringa oleifera seed oil variety Periyakulam 1. J Food Comp Anal 2002;15:79-101.
5. Mingrone G, Greco AV, Finotti E, Passi S. Free fatty acid: a stimulus for mucin hypersecretion in cholesterol gallstone biles. Bioch Bioph Acta 1988;958:52-9.
6. Coseteng MY, Lee CY. Changes in apple polyphenolxidase and poly-

Tabella 1. Valori degli acidi grassi, espressi in percentuale, dell'olio di moringa a confronto con altri oli alimentari.

Acidi grassi	Moringa	Oliva	Arachidi	Palma	Girasole	Mais	Burro	Strutto
C16:0	12.12	20.97	9.91	41.21	5.92	11.69	20.86	24.02
C16:1	1.87	1.85	0	0.37	0.27	0.4	1.9	2.55
C18:0	10.27	2.28	2.53	4.34	4.74	2.3	9.4	16.67
C18:1	62.50	69.59	51.3	38.45	32.91	29.88	20.68	39.06
C18:2	1.35	5.31	27.87	9.28	49.89	49.83	1.57	8.95
C18:3	0.00	0.00	0	3.3	0.33	0.6	1.18	0.92
C20:0	0.00	0.00	2.28	0.4	0.54	0.4	0	0
C20:4	0.00	0.00	0	0	0	0	0	1.83
C22:0	5.83	0	3.25	0	0	0	0	0
Sat/Ins	0.43	0.30	0.23	0.89	0.13	0.18	1.19	0.76
Insat. Grade	0.67	0.82	1.07	0.67	1.34	1.32	0.29	0.70
Sat/Inst.grade	42.09	28.32	16.79	68.30	8.36	10.92	25.33	58.47

Tabella 2. Contenuto in polifenoli totali e capacità antiossidante dell'olio di moringa a confronto con altri alimenti.

Prodotti	Ka/Kc	Polifenoli Tot. (mg/g)
Moringa	3,03	52,2
Olio extra vergine d'oliva	0,3	51
Radicchio	4,34	13,9
Mela	0,09	4,08
Pere	0,34	2,7
Cioccolato latte	1,47	8,54
Cioccolato fondente	1,98	18,59
Grano duro	0,6	0,31
The Bianco	6,53	0,62
Vino Cabernet-Souvignon	7,94	2,72
Vino Nero D'Avola	12,23	3,11
Vino Syrha	6,32	3,01
Vino Merlot	16,46	3,03

phenol concentration in relation to degree of browning. J Food Sci 1987;52:985-6.
7. Finotti E, D'Ambrosio M, Paoletti F, et al. Synergistic effects of α-tocopherol, β-sitosterol and squalene on antioxidant activity assayed by crocin bleaching method. Nahrung 2000;44:373-4.
8. Di Majo D, Giammanco S. Composti antiossidanti del vino nell'alimentazione. Palermo: ILa Palma; 2010.

Impact of a single, intense prenatal stress on ethanol drinking behaviour and cognition in adult male rats

G. Gambino, F. Plescia, A. Brancato, R.A.M. Marino, C. Cannizzaro

Department of Sciences for Health Promotion and Mother and Child Care Giuseppe D'Alessandro, University of Palermo, Italy

Introduction

Early exposure to stressful stimuli is crucial for developing varied behavioural patterns in adulthood such as anxiety, cognitive dysfunction and abuse disorders.[1] The alteration of the hypothalamic–pituitary-adrenal (HPA) axis represents the neurobiological substrate responsible of the behavioural consequences of prenatal stress (PS). Indeed, prenatal manipulation of the HPA axis impacts on cognitive performance of the adult offspring,[2] but also on vulnerability to alcohol consumption.[3] Prenatal acute, moderate restraint stress has proved to facilitate HPA axis development of the offspring, since maternal corticosterone secretion leads to the reduction of anxiogenic behaviour and an improvement in the ability to cope with stress, increasing both the rate of learning[2] and long-term potentiation,[4] but also gestational stress blunts initial alcohol-induced HPA axis activation.[3] In our recent study, moderate alcohol intake proved to reduce emotionality and facilitate the adaptive responses to stress, enhancing behavioural flexibility.[5] Based upon these findings, we aimed at assessing the impact of a single, intense prenatal stress on exposure to alcohol preference and on the effects exerted by ethanol on behavioural reactivity, anxiety-like behaviour and spatial learning in adult male Wistar rats.

Materials and Methods

36 adult male Wistar rats were separated into three groups: prenatally stressed, alcohol free- access (PS-FA); free access (FA) and control group (CTR).

Prenatal stress procedures involved immobilization of pregnant dams for 120 minutes on Gestational Day 16.[6] Male adult PS-FA and FA offspring were subjected to a three-bottle choice paradigm with free access to ethanol 10% (v/v), white wine (Tavernello 11 v%, Italy, diluted with water until 10 % v/v) and water, along a four-week period. The volumes consumed were recorded daily.

To assess the influence of ethanol self-administration on behavioural patterns we used respectively: the open field test (OFT) for behavioural reactivity, the elevated plus-maze test (EPM) for anxiety-like behaviour and the Morris Water Maze (MWM) test for spatial learning.

Results and Discussion

Our results showed that there is no statistical difference in ethanol consumption between PS-FA and FA rats, both consuming moderate doses (3.00±0.8 g/kg/day), apart from the first week when the intake was higher.

Results from the OFT and EPM displayed a reduction in anxiety-like behaviour in PS-FA and FA rats, when compared to CTR. Also, PS-FA group further displayed a reduced anxiety-like behaviour, compared to FA.

Indeed, the OFT showed a significant increase in the number of transitions in the center of the arena ($P<0.0005$) and amount of time spent on the center of arena ($P<0.0073$) in FA rats, compared with controls. The time spent on the center of arena was significantly increased ($P<0.0318$) in PS-FA, compared with FA rats.

Data from the EPM showed a significant increase in the percentage of time spent ($P<0.045$) and of the number of entries ($P<0.0001$) in the open arms of the FA group, compared with CTR. No significant differences was found between PS-FA group, compared with FA.

Finally, PS-FA and FA rats displayed a significant reduction in latencies and distance travelled to find the platform in the place learning of the MWM ($P<0.001$), with respect to controls. In particular, PS-FA group showed significant improvements in the MWM ($p<0.01$), when compared to FA.

Conclusions

Our study proved that both PS-FA and FA groups had an irregular trend in alcohol consumption, representing an initial binge-like drinking behaviour, then ensued by a voluntary reduction in alcohol intake to moderate values,[5,7] evidencing that prenatal stress does not influence ethanol consumption in adulthood.

Moderate ethanol intake exerts anxiolytic properties, as showed by the OFT and EPM, improving the response to stress in the adversative situation in the MWM. Furthermore, ethanol facilitating effect on cognitive performance was enhanced by prenatal attenuation of HPA axis. Indeed, corticosterone levels are inversely correlated with mechanisms of hippocampal neuroplasticity such as BDNF release.[8]

Correspondence: Carla Cannizzaro, Department of Sciences for Health Promotion and Mother and Child Care Giuseppe D'Alessandro, University of Palermo, via del Vespro 129, 90127 Palermo, Italy.
E-mail: carla.cannizaro@unipa.it

Journal of Biological Research 2015; 88:5161

In conclusion, our findings further highlight the role of prenatal experiences on ethanol-induced mechanisms of neuroadaptation, indicating that a single, intense stress during early gestational period interacts with alcohol effects in adulthood.

References

1. Weinstock M. The potential influence of maternal stress hormones on development and mental health of the offspring. Brain Behav Immunol 2005;19:296-308.
2. Cannizzaro C. Single, intense prenatal stress decreases emotionality and enhances learning performance in the adolescent rat offspring: interaction with a brief, daily maternal separation. Behav Brain Res 2006;169:128-36.
3. Van Waes V. Hypo-response of the hypothalamic-pituitary-adrenocortical axis after an ethanol challenge in prenatally stressed adolescent male rats. Eur J Neurosci 2006;24:1193-200.
4. Fujioka A. Differential effects of prenatal stress on the morphological maturation of hippocampal neurons. Neuroscience 2006;141:907-15.
5. Cacace S. Evaluation of chronic alcohol self-administration by a 3-bottle choice paradigm in adult male rats. Effects on behavioural reactivity, spatial learning and reference memory. Behav Brain Res 2011;219:213-20.
6. Pacak K. Adrenalectomy augments in vivo release of norepinephrine in the paraventricular nucleus during immobilization stress. Endocrinology 1993;133:1404-10.
7. Juárez J, Barrios de Tomasi E. Sex differences in alcohol drinking patterns during forced and voluntary consumption in rats. Alcohol 1999;19:15-22.
8. Radecki DT. BDNF protects against stress-induced impairments in spatial learning and memory and LTP. Hippocampus 2005;15:246-53.

Anti-inflammatory effects of Sicilian pistachio (*Pistacia vera* L.) nut in an *in vitro* model of human intestinal epithelium

C. Gentile, A. Perrone, A. Attanzio, A.M. Pintaudi, L. Tesoriere, M.A. Livrea

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

Intestinal epithelial cells play an important role in the mucosal inflammatory response. These cells synthesize and secrete inflammatory mediators, and selectively modulate the permeability of the epithelial monolayer thus exposing immune cells to antigens. Although intestinal inflammatory response is crucial to maintain gut structural integrity and function, alteration and dysregulation of inflammatory pathways contribute to tissue damage and ulceration, and are thought to be pivotal factors in the pathogenesis of different inflammatory gut diseases.[1] The limited efficacy of conventional pharmacological therapy in the intestinal inflammatory conditions has fostered research on alternatives and, at the same time, stresses the importance of prevention. In this context, the influence of dietary components, becoming in a physiological close proximity to intestinal cells and then to inflammatory processes within the intestinal mucosa, appears of nutritional and clinical interest.[2] Among natural preventive and complementary approaches to improve inflammatory symptoms, dietary polyphenols represent potential candidates and proanthocyanidins are particularly interesting.[3] For their relatively high concentration in a number of edible plants and their high digestive stability and limited intestinal adsorption,[4,5] proanthocyanidins reach the colon at relatively high concentrations and may have direct effects on the intestinal mucosa through their interaction with the intestinal epithelial cell membranes.[6,7]

The edible pistachio nut has been ranked among the first 50 food products highest in antioxidant potential.[8] A number of data show that the pistachio nut consumption has positive effects in human serum lipid profile and cardiovascular disease (CVD) risk factors[9,10] and significantly improves oxidative status and reduces circulating inflammatory biomarkers.[11] Our previous research provided evidence that a hydrophilic extract from Sicilian pistachio nuts (HPE) contains substantial amounts of polyphenols, including proanthocyanidins, and possesses radical scavenging and antioxidative properties in *in vitro* models of lipid oxidation.[12] Moreover we also demonstrated that HPE has anti-inflammatory activities in lipopolysaccharide (LPS)-activated macrophages interfering with the NF-*k*B activation, and that the high molecular weight proanthocyanidin fraction (PF) can play a major role as the bioactive component of HPE.[13]

In the present study we investigated the activity of HPE, and of its polymeric proanthocyanidin fraction as well, in an *in vitro* model of intestinal inflammation, consisting of Caco-2 cells differentiated into epithelial intestinal cells and exposed to the inflammatory actions of interleukin (IL-1β). Our results clearly show that HPE effectively inhibits the inflammatory response in intestinal epithelial cells, and that highly polymeric proanthocyanidin components exhibit qualitative and quantitative effects substantially comparable to those of whole extracts when tested at the same concentration found in the extracts. The protective effects are expressed through a marked decrease in release and expression of inflammatory mediators and occur in parallel with a reduced activation of the nuclear factor-1β. Moreover, our results clearly show that HPE can partially prevent the IL-1β-induced gap formation with perturbation of the monolayer integrity, as shown by a limited IL-1β-induced increase of paracellular permeability. Finally we provide evidence that HPE treatment increases transepithelial electrical resistence of Caco-2 cells monolayer, demonstrating that protective effects of HPE under our conditions occur in parallel with molecular interaction of nut components with the epithelial cells membranes.

To assess the physiological relevance of the tested concentration it is worth noting that a single serving of pistachio nut (28.34 g) (USDA National Nutrient Database for Standard Reference) contains around 62,34 mg polymeric proanthocyanidins (cyanidin equivalents). Once diluted in a gastrointestinal volume of 600 mL, this result in a 3.5 µM concentration (104 µg/mL) (as cyanidin equivalents) of polymeric proanthocyanidins which represent a plausible concentration in the human gut.[14] This concentration is one order higher than the concentrations selected in our cell model and this suggests that our results might be physiologically relevant in the gastrointestinal tract.

Data here presented may further remark the potentially beneficial health effects that may arise by daily intake of small quantity of pistachio nut. Widely available, inexpensive and frequently consumed, this nut for its favorable fatty acid profile and high content in bioactive antioxidant compounds, positively influences the plasma lipid parameters and oxidative status, and elicits antinflammatory properties. In this respect high content in large proanthocyanidins consumption of pistachio nut can exerts locally significant beneficial effects to physiology of gastrointestinal tract.

Correspondence: Carla Gentile, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze, Ed. 16, 90127 Palermo, Italy.
E-mail: carla.gentile@unipa.it

Journal of Biological Research 2015; 88:5161

References

1. Mayer L. Evolving paradigms in the pathogenesis of IBD. J Gastroenterol. 2010;45:9-16.

2. Netsch MI, Gutmann H, Aydogan C, Drewe J. Green tea extract induces interleukin-8 (IL-8) mRNA and protein expression but specifically inhibits IL-8 secretion in caco-2 cells. Planta Med 2006;72:697-702.
3. Shapiro H, Singer P, Halpern Z, Bruck R. Polyphenols in the treatment of inflammatory bowel disease and acute pancreatitis. Gut 2007;56:426-35.
4. Deprez S, Mila I, Huneau JF, et al. Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. Antioxid Redox Signal 2001;3:957-67.
5. Rios LY, Bennett RN, Lazarus SA, et al. Cocoa procyanidins are stable during gastric transit in humans. Am J Clin Nutr 2002;76:1106-10.
6. Erlejman AG, Jaggers G, Fraga CG, Oteiza PI. TNFalpha-induced NF-kappaB activation and cell oxidant production are modulated by hexameric procyanidins in Caco-2 cells. Arch Biochem Biophys 2008;476:186-95.
7. Oteiza PI, Erlejman AG, Verstraeten SV, et al. Flavonoid-membrane interactions: a protective role of flavonoids at the membrane surface? Clin Dev Immunol 2006;12:19-25.
8. Halvorsen BL, Carlsen MH, Phillips KM, et al. Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. Am J Clin Nutr 2006;84:95-135.
9. Edwards K, Kwaw I, Matud J, Kurtz I. Effect of pistachio nuts on serum lipid levels in patients with moderate hypercholesterolemia. J Am Coll Nutr 1999;18:229-32.
10. Kocyigit A, Koylu AA, Keles H. Effects of pistachio nuts consumption on plasma lipid profile and oxidative status in healthy volunteers. Nutr Metab Cardiovasc Dis 2006;16:202-9.
11. Sari I, Baltaci Y, Bagci C, et al. Effect of pistachio diet on lipid parameters, endothelial function, inflammation, and oxidative status: a prospective study. Nutrition. 2010;26:399-404.
12. Gentile C, Allegra M, Angileri F, et al. Polymeric proanthocyanidins from Sicilian pistachio (Pistacia vera L.) nut extract inhibit lipopolysaccharide-induced inflammatory response in RAW 264.7 cells. Eur J Nutr 2012;51:353-63.
13. Gentile C, Tesoriere L, Butera D, et al. Antioxidant activity of Sicilian pistachio (Pistacia vera L. var. Bronte) nut extract and its bioactive components. J Agric Food Chem 2007;55:643-8.
14. Fraga CG, Galleano M, Verstraeten SV, Oteiza PI. Basic biochemical mechanisms behind the health benefits of polyphenols. Mol Aspects Med. 2010;31:435-45.

Development of smart probiotics against *Clostridium perfringens*

T. Gervasi,[1,2] M.J. Mayer,[1] R.B. Lo Curto,[2] G. Dugo,[2] A. Narbad[1]

[1]Institute of Food Research, Norwich Research Park, Norwich, UK; [2]Department of Food and Environmental Sciences, University of Messina, Italy

Probiotics are living microorganisms which confer health benefits to the host. Our work was initially focused on investigating the possibility of producing *smart* probiotics, which are probiotics with modified extra functions, such as the heterologous expression of an antimicrobial. Our first aim was to identify antimicrobial activities or agents which could act against *Clostridium perfringens. C. perfringens*, one of the most pathogenic species in the *Clostridium* genus, is causing increasing concern because it is responsible for severe infections both in human and animals, especially poultry. It is considered the third leading cause of food poisoning death in the UK and USA and causes necrotic enteritis (NE) in poultry.[1] Bacteriophages and their endolysins have been used to treat human infections and to control antibiotic-resistant pathogenic bacteria in animal models.[2-4] Bacteriophages infecting *C. perfringens* are both lysogenic and virulent and show either long tails if members of the Siphoviridae family or short tails if members of the Podoviridae, both in the order of Caudovirales. Several putative bacteriophage endolysins have been identified, both from *C. perfringens* bacteriophages and by genome mining, producing a rich resource of enzymes.[5] The use of endolysins as antimicrobials has been explored. In fact recent studies showed the efficiency of these proteins in killing or controlling pathogenic bacteria when used alone, by a synergistic action with antibiotics or also in combination with other proteins such as the holin. The first test conducted to observe the presence of bacteriophages which contain an endolysin, was a plaque assay test. The appearance of a plaque is the oldest, but at the same time the most useful and direct confirmation way of a phage presence.[6] The nutrient agar layer method was first described by Gratia to enumerate phage particles.[7] A thin layer of soft agar, containing host bacteria and bacteriophages, is poured on a thick layer of higher concentrated agar, used as nutrient medium by bacteria. The phages infect the bacteria and after the production of new phage particles, which are released after bacterial lysis, start a new infectious cycle. To investigate the presence of prophages in *C. perfringens* strains, bacteriophage release was induced by mitomycin C. The supernatants were then concentrated by PEG precipitation then both observed by TEM and used for plaque assays. In the anaerobic cabinet 25 µl aliquots of filtered mitomycin C-induced supernatant were spotted on plates of BHI agar which had been overlaid with 4 ml BHI top agar (0.7% agar) seeded with 100 µL of *C. perfringens* overnight culture. Plates were incubated for up to 48 h and checked regularly for plaque formation. TEM observations on PEG-precipitated supernatants obtained after bacteriophage induction showed the presence of bacteriophages both in *C. perfringens* strains 54116-97 and 6081-97 (Figure 1). The bacteriophages in these supernatants did not produce plaques on any of the 25 strains tested. However, the mitomycin C-induced supernatant from strain 6081-97 did show antimicrobial activity on several *C. perfringens* strains, evident because of the zones of clearance around the supernatant dropped on plates (Figure 2). This activity appeared to be variable so to elucidate this behavior, strain 6081-9736 was streaked close to other *C. perfringens* strains such as 562118-98, 4519-98, 2151-88, and DP3. Bacteria which were potential producers of antimicrobials were streaked across BHI agar plates and potential sensitive strains were cross-streaked at a 90° angle with regard to the first streak of the indicator organism or parallel to this one and incubated overnight. The aim was to check if this strain was able to inhibit the growth of other *C. perfringens* strains. Among the strains tested, strain 2151-88 was shown to be able

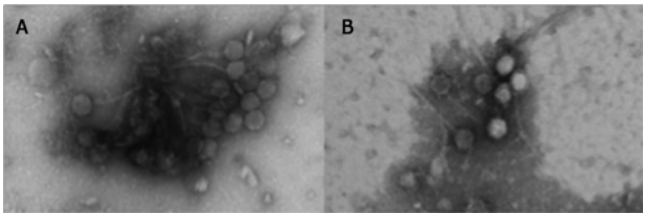

Figure 1. Tailed bacteriophages found in mitomycin-C induced supernatants of strain 6081-97 (A) and 5416-97 (B).[10] Scale bar represents 100 nm.

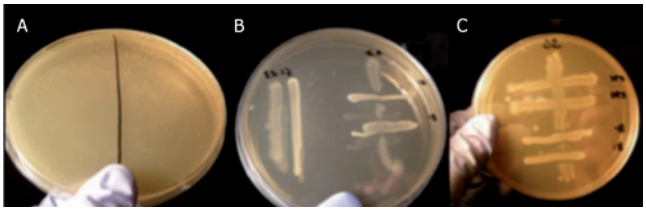

Figure 2. A) Antimicrobial effect of *C. perfringens* 6081-97 mitomycin-C induced supernatant on *C. perfringens* NCTC3110 and (B-C) on *C. perfringens* 2151-88 and 6081-97.

Correspondence: Teresa Gervasi, Department of Food and Environmental Sciences, University of Messina, viale Ferdinando Stagno D'Alcontres 31, 98166 Messina, Italy.
E-mail: tgervasi@unime.it

Journal of Biological Research 2015; 88:5161

to inhibit the growth of strain 6081-97 (Figure 2B,C), but its antimicrobial activity was not constant in repeat tests. In the same way strain 5416-97 showed an antimicrobial activity against other *C. perfringens* strains, but again with non-constant responses. It has been previously reported that mitomycin C can induce the production of bacteriocins from *C. perfringens*.[8] The genomes of both *C. perfringens* strains have been sequenced using Illumina technology and are currently being mined for genes associated with bacteriocin production. Further studies are in progress to investigate bacteriocin production and to assess the meaning of the observed variability in the antimicrobial activity of *C. perfringens* strains tested. The genome sequencing also allowed the identification of an active endolysin against *C. perfringens* from strain 5146-97[9] and we will investigate the possibility of a further lysin from strain 6081-97.

References

1. Keyburn AL. NetB, a new toxin that is associated with avian necrotic enteritis caused by Clostridium perfringens. PLoS Pathog 2008;4:26.
2. Fischetti VA. Bacteriophage lytic enzymes: novel anti-infectives. Trends Microbiol 2005;13:491-6.
3. Fischetti VA. Bacteriophage lysins as effective antibacterials. Curr Opin Microbiol 2008;11:393-400.
4. Fischetti VA. Bacteriophage endolysins: a novel anti-infective to control Gram-positive pathogens. Int J Med Microbiol 2010; 300:357-62.
5. Schmitz JE. Lytic enzyme discovery through multigenomic sequence analysis in Clostridium perfringens. Appl Microbiol Biotechnol 2011;89:1783-95.
6. Gallet R, Kannoly S, Wang IN, Effects of bacteriophage traits on plaque formation. BMC Microbiol 2011;11:181.
7. Gratia A. Numerical relations between lysogenic bacteria and particles of bacteriophage. Ann Inst Pasteur 1936;57:652-76.
8. Mahony DE. Induction of bacteriocins from Clostridium perfringens by treatment with mitomycin C. Antimicrob Agents Chemother 1977;11:1067-8.
9. Gervasi T, Horn N, Wegmann U, et al. Expression and delivery of an endolysin to combat Clostridium perfringens. Appl Microbiol Biotechnol 2014;98:2495-505.
10. Gervasi T, Lo Curto RB, Narbad A, Mayer M. Complete genome sequence of CP51, a temperate bacteriophage Of Clostridium perfringens. Arch Virol 2013;158:2015-7.

How cognitive behavioural therapy can modulate negative emotional factors as anxiety or depression in obese patients

G. Giammanco, M. La Guardia, S. Martorana, D. Di Majo, M. Giammanco

Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo

Emotional factors have great influence on eating habits and increase the risk of obesity.[1] Few studies demonstrate that patients affected by binge eating disorder felt negative emotions before overeatings; the main emotions reported were anxiety, then sadness and tiredness. Emotional eating is related to the stress,[2,3] so when subjects feel anxiety and depression, is more probable that they use food as modulator of their emotions, in this way the weight develops fast with all risks of obesity. The obesity is often attended by a specific syndrome called metabolic syndrome; the main symptoms are insulin resistence, hyperinsulinemia, not-insulin-dependent diabetes mellitus, dyslipidemia, central obesity, hyperuricemia, hypertension; this clinic condition predisposes to cardiovascular diseases.[4] So the aim of our study is to observe how CBT can help to manage negative emotions as anxiety e depression[5] and in this way modulate the emotional eating. We began to select a sample of obese subjects, and gave them few questionnaires to evaluate the degree of anxiety and depression (STAI/BDI), the level of self-esteem (BASIC SE), and if there was a diagnosis of Binge Eating Disorder (BED) by a Binge Eating Scale (BES). The first results show a significant relation between anxiety and depression, low level of self-esteem, and their relation with BED. We analyzed the results after an year of treatment with CBT and dietotherapy and we found that patients without BED had a more significant loss of weight. In the subjects with BED the binges disappeared and the self-esteem increased.

References

1. Dallman MF. Stress-induced obesity and the emotional nervous system. Trends Endocrinol Metab 2010;21:159-65.
2. Masheb RM, Grilo CM. Emotional overeating and its associations with eating disorder psychopatology among overweight patients with Binge Eating Disorder. Int J Eating Dis 200;39:141-6.
3. Gluck ME. Stress response and binge eating disorder. Appetite 2006;46:26-30.
4. Reaven GM. Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.
5. Baumesteir RF, Campbell GD, Krueger JD, Vohs KD. Does high self-esteem cause better performance, interpersonal success, happiness, or healthier lifestyle? Psyc Sci Pub Int 2003;4:1-44.

Correspondence: Giulia Giammanco, Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, via delle Scienze ed. 16, 90127 Palermo, Italy.
E-mail: giugia3@tin.it

Evaluation of plasma antioxidant status after red wine intake

M. Giammanco, M. La Guardia, S. Martorana, D. Di Majo

Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo

Abstract

Several studies report that the plasma antioxidant capacity (PAC) increases after a single ingestion of red wine. However, data on the different behaviour of PAC after food intake in subjects of the same sex are still lacking. On the basis of these observations some investigations were undertaken in order to evaluate the effects of red wine intake on PAC in a homogeneous groups of healthy female volunteers (n=18). Additionally, the possible correlation between increase of PAC values and increase in uric acid levels was also assessed. PAC and uric acid concentrations were determined before wine intake and 50, 120 and 240 minutes thereafter. The results obtained following these studied highlighted two different patterns of variation of plasma AC values after red wine intake in women. Group A exhibits a significant increase in PAC at 120 minutes after wine consumption while Group B showed a peak level of AC 50 minutes after wine intake. However, no significant correlation was highlighted between increased levels of uric acid and PAC. These results provide a strong argument for the hypothesis that sampling procedures may be one of the confounding factor in studies on the plasma antioxidant status after food or beverage consumption. These preliminary observations indicate that sex-based selection of volunteers should be considered in further investigations.

Introduction

Experimental, clinical and epidemiologic, observations report that the consumption of flavonoid-rich food is associated with a lower incidence of heart disease, ischemic stroke, cancer and other chronic diseases.[1] Red wine is known to be a source of polyphenolic compounds with antioxidant properties.[2] Plasma antioxidant capacity (PAC) is a good parameter of the antioxidant status.[3] Total antioxidant capacity (TAC) represents the non-enzymatic antioxidants network. TAC comprises the cumulative effects of the whole antioxidants molecules present in plasma matrix.

Correspondence: Marco Giammanco, Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, via Elia 3, 90127 Palermo, Italy.
E-mail: marco.giammanco@unipa.it

Journal of Biological Research 2015; 88:5161

Materials and Methods

Eighteen healthy female volunteers, aged 24-32 years were recruited among the laboratory staff. These subjects were non-smokers and no habitual alcohol consumers. All these subjects were normolipidemic and with a regular ovulatory cycle. None of these volunteers had previous cardiovascular, hepatic, gastrointestinal or renal diseases. Blood samples were obtained before the wine intake (baseline value) and then 50, 120, 240 minutes after wine consumption respectively. *Analysis of Plasma Antioxidant Capacity by the Crocin Bleaching Assay* (CBA) is a competition kinetics assay method for measuring the antioxidant capacity of individual compounds, plant extracts, or plasma.[4-6]

Results

The results identified two groups according to the trend of the PAC values observed after red wine intake. Group A included ten subjects while Group B consisted of eight subjects. The statistic analysis of the data show that, in Group A, PAC levels, determined before wine intake were significantly different ($P<0.01$) from those measured 120 min after wine ingestion. The rate of increase was 69% after 120 min from red wine intake. However, in this group no significant difference ($P>0.05$) was observed in the value determined after 50 minutes as compared to baseline value. PAC remained significantly elevated after 240 minutes of red wine intake. On the other hand, in group B the highest values were observed after 50 minutes from red wine intake ($P<0.01$). PAC levels increased of 77.6% at 50 min as compared to the baseline value. These levels significantly decreased 120 min after wine consumption ($P=0.01$) and then returned to baseline values after 240 min.

Discussion

The present study shows that the intake of red wine induce a time-dependent increase of the PAC in healthy female subjects. Interestingly, our results highlight that, in these subjects, two main different time-dependent pattern of variations of plasma antioxidant values can be observed following a red wine intake. In fact, in a group

of ten subjects (Group A) the maximum antioxidant capacity value is reached later than in the other eight subjects (Group B). These results may be also, explained with the possible interaction of some polyphenols, present in the red wine with the circulating sex-hormone binding globulin (hSHBG). On the basis of these considerations it is conceivable to hypothesize that the different trend of antioxidant capacity after red wine intake we observed may be due to the different levels of circulating sex hormones related to the phase of the ovarian cycle in which the woman was at the time of the study.

Conclusions

The present study show that the intake of red wine induce a time dependent increase of the plasma antioxidant activity in healthy female subjects. Moreover, these observations indicate that further investigations with a wide number of subjects are needed to better assess the relationship between trend of sex hormones levels during the three phases of the ovarian cycle and variations of the antioxidant status after the consumption of rich-in-antioxidants foods.

References

1. Bosetti C. Flavonoids and breast cancer risk in Italy. Cancer Epid Bio Prev 2005;144:805-8.
2. Rice-Evans C. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad Biol Med 1996;20:933-56.
3. Ghiselli A. Beer increases plasma antioxidant capacity in human. J Nutr Biochem 2000;11:76-80.
4. Di Majo D. Flavanones in citrus fruit: structure-antioxidant activity relationships. Food Res Int 2005;38:1161-6.
5. Di Majo D. The antioxidant capacity of red wine in relationship with its polyphenolic costituents. Food Chem 2008;111:45-9.
6. Ordoudi SA. Crocin bleaching assay step by step: observations and suggestion for an alternative validated protocol. J Agr Food Chem 2006;54:1661-3.

Does mating group size negatively affect female investment in the simultaneous hermaphrodite *APLYSIA punctata*?

P. Gianguzza,[1] L. Angeloni[2]

[1]Department of Earth and Sea Sciences, University of Palermo, Italy; [2]Department of Biology, Colorado State University, Fort Collins, CO, USA

Theoretical models and empirical studies have supported the idea that hermaphroditic sex allocation is sensitive to social conditions.[1,2] In general, when the mating group size equals two, both partners are expected to allocate as few resources as possible to sperm production, just enough to fertilize each other's ova, and devote all excess energy to egg production.[1] When the average number of possible partners increases, sperm competition for fertilization leads to greater investment in sperm, and resource allocation to female function is expected to decrease. However to our knowledge the relationship between mating group size and sexual behavior has been investigated in only a few species of outcrossing marine hermaphrodites.

The research presented here explores whether the sea hare *Aplysia punctata* (Cuvier, 1803), a simultaneous hermaphrodite, adjusts its investment in female function in response to social conditions. We experimentally manipulated the group size of mature *A. punctata* to determine whether individuals diminish female investment under different social conditions. We predicted that individual *A. punctata* would diminish egg mass production in larger social groups.

A total of 200 adult *A. punctata* (from 40 to 50 mm long) were collected within the rocky shore of the Ustica Island (Cala Sidoti 038°42.50N; 013°9.00E) from January through March 2006. After collection, sea hares were isolated over a period of 30 days in 35 L aquaria with circulating natural seawater, at ambient light (12 hours light:12 hours dark) and temperature regimes (22°C). *Ulva rigida* (C.Agardh 1823) was supplied daily *ad libitum*, to ensure continuous access to food.

A laboratory experiment was carried out in order to determine if egg mass production of *A. punctata* is related to the size of the social group. Following isolation, animals were randomly assigned to a group of two (P), three (T), four (Q) and eight (O) animals, with 10 replicates of each group size for a total of 170 animals, and constantly monitored for one month. These groups were maintained in tanks (50 cm in diameter, 40 cm in deep) filled with running seawater at a stable temperature and controlled light regime as described previously. All treatments were performed simultaneously and no *A. punctata* was used more than once to ensure the independence of data.

Following the treatments, each individual that had been observed mating as a sperm recipient was isolated and monitored for an additional 30 days in separate cages with continuous access to food. Every day we checked for the presence of a spawned egg mass. When an egg mass was detected, it was gently removed from the cage and its wet weight was recorded to the nearest 0.01 g. The proportions of egg masses showing no development (non viable eggs) and normal development (viable eggs) were determined for each egg mass. Differences in number and width of egg masses (a measure of female investment) were analyzed by two separate one-way Analyses of Variance (ANOVA), with *mating group* (MG), including its four levels (P, T, Q, O) as a fixed factor. Data were tested for normality with a Bartlett test. Homogeneity of variances was also checked with Cochran's C-test. Following the ANOVA, means were compared (at α=0.05) with Student-Newman-Keuls (SNK) tests. The GMAV 5·0 software (University of Sydney, Australia) was used to perform the statistical tests.

All egg masses produced subsequent to the experiment contained fertilized eggs indicating that exogenous sperm transfer had been successful. Egg masses were always laid by individuals that were observed to be the first member of a mating chain (the terminal sperm recipient) and never by an animal that acted as both sperm recipient and sperm donor at the same time. We observed that *A. punctata* laid fertilized egg masses during copulation, after few hours or, at most, 36 hours after copulation.

A total of 23 egg masses were produced: 10 in the P treatment (1 from each pair), 3 in the T treatment (2 from individuals that mated in a pair and 1 from a chain of three), 4 in the Q treatment (all from individuals that mated in a pair) and 4 in the O treatment (2 from individuals that mated in a pair,1 from a chain of three and 1 from a chain of four). All egg masses showed normal cleavage, development of embryos and the hatching of planktonic larvae within 7-10 days. Group size strongly influenced egg production ($F_{3,39}$=6.15; P=0.0017) and SNK results revealed that the number of spawned egg masses was greater for the P treatment than any other treatment (P>T=Q=O) and ranged from a maximum of 1.2±0.1 egg masses per individual (SE) (laid by individuals from P treatments; Figure 1) to a minimum of 0.3±0.1 (SE) (laid by individuals from T and O treatments; Figure 1). Moreover, egg mass size varied significantly as a function of the social condition ($F_{3,39}$=8.26; P=0.0003). In particular, SNK results showed that egg mass width was greater for the P treatment than any other treatment (P>T=Q=O) and ranged from a maximum of 26±3.8 (SE) mm (laid by individuals in the P treatment) to a minimum of 8.0±3.8 (SE) mm (laid by individuals in the T treatment).

Our findings showed that group size strongly influenced female function and that number and width of egg masses were greater for individuals maintained in pairs than for those maintained in larger social groups. These results indicate that *A. punctata* respond as

Correspondence: Paola Gianguzza, Department of Earth and Sea Sciences, University of Palermo, via Archirafi 22, 90123 Palermo, Italy.
Tel: +39.091.23862857.
E-mail: paola.gianguzza@unipa.it

Journal of Biological Research 2015; 88:5161

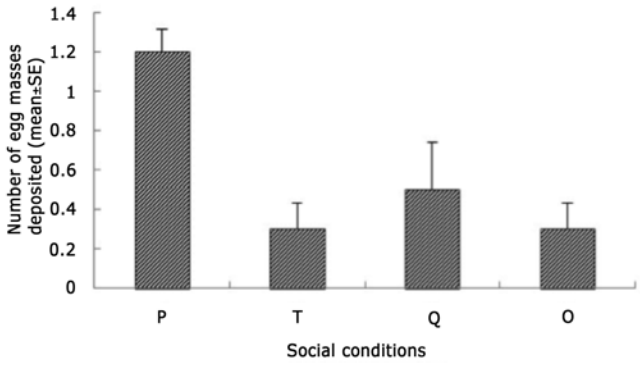

Figure 1. Egg masses laid by individuals from groups of two (P), three (T), four (Q) and eight (O) animals.

expected according to sex allocation theory for simultaneous hermaphrodites.[3] Furthermore, our experiments reveal that female allocation in this species is phenotypically flexible, as fully mature individuals were able to adjust their investment in the number and size of egg masses with the size of the social group.

References

1. Charnov EL. Simultaneous hermaphroditism and sexual selection. P Natl Acad Sci 1979;76:2480-4.
2. Charnov EL. The theory of sex allocation. Princeton, NJ: Princeton University Press; 1982.
3. Michiels NK. Mating conflicts and sperm competition in simultaneous hermaphrodites. In: A.P. Møller (ed.), Sperm competition and sexual selection. London: Academic Press; 1998. pp. 219-54.

Allevamento a ciclo biologico naturale di *Cornu aspersum* (Müller, 1774): proprietà alimentari e usi del secreto o bava di lumaca

G. Russo, F. Grisafi

Dipartimento di Scienze Agrarie e Forestali, Università di Palermo, Italy

Cornu aspersum (Muller, 1774), specie che in passato era attribuita al genere *Helix* (*Helix aspersa*), è un mollusco gasteropode terrestre appartenente alla famiglia degli Helicidae è diffuso nel bacino del Mediterraneo e nell'Europa nord-occidentale. In Italia è presente in tutta la penisola, isole comprese. Il diametro della sua conchiglia può arrivare a 35 mm., con un'altezza di 27-31mm; il suo peso è di circa 13-15 g. La colorazione di fondo va dal verdastro al giallastro; può essere uniforme, ma più spesso sono presenti da 1 a 5 bande spiralate marroni, con screziatura gialla o bianca. Come molte chiocciole, *C. aspersum* è ermafrodita, cioè ogni individuo possiede sia organi riproduttivi maschili che femminili; non è tuttavia in grado di autofecondarsi (ermafroditismo insufficiente).

L'azienda elicicola *La Lumaca del Belice* sita nel territorio di Sambuca di Sicilia (AG) su una superficie di circa 10.000 mq, usa un sistema di allevamento a ciclo naturale biologico in campo aperto che dà un aumento notevole della produzione per metro quadrato ma soprattutto dà la possibilità di portare le chiocciole alle caratteristiche ottimali nel più breve tempo possibile. Con questo sistema di allevamento le chiocciole nascono e crescono dentro lo stesso recinto, evitandosi i lavori di spostamento dei piccoli causa di mortalità elevata dovuta allo stress provocato dal cambio di habitat ed allo schiacciamento durante il trasferimento a causa della fragilità del tenero guscio che le protegge. Il ciclo di allevamento inizia con la coltivazione di bietola e cavolfiore per l'alimentazione e di trifoglio per ricreare l'habitat naturale nei mesi di marzo/aprile, fino ad arrivare al mese di novembre quando la raccolta delle chiocciole sarà completata ed il terreno verrà preparato per il successivo ciclo. Durante questo periodo gli ortaggi non verranno trattati né con concimi chimici né con pesticidi. Le prime chiocciole riproduttrici verranno immesse nei recinti nel primo periodo primaverile, quando la vegetazione avrà raggiunto le caratteristiche ottimali di vegetazione. Qui le chiocciole si accoppieranno e concepiranno le nuove chiocciole che, già nei primi mesi di settembre-ottobre, avranno raggiunto le caratteristiche ottimali di pezzatura e peso e saranno pronte per essere commercializzate. Diversi studi hanno contributo ad approfondire le conoscenze utili ai fini della valorizzazione del prodotto alimentare *lumaca*, non solo in termini alimentari ma anche economico-sociali in virtù della crescente richiesta del prodotto da parte del mercato interno e della prospettiva di poter integrare, attraverso l'attività di allevamento del mollusco, l'attività agricola. L'indagine analitica finalizzata all'approfondimento conoscitivo delle proprietà dietetico-nutrizionali di cinque diverse specie di molluschi polmonati del genere *Helix* di interesse alimentare, condotta da Novelli[1] permette di trarre alcune considerazioni. Il contenuto in sostanza secca della parte edibile è prossimo al 20%, i due-terzi del quale sono rappresentati da proteine. Il contenuto lipidico è decisamente ridotto (inferiore all'1%) e ciò non è sorprendente in ragione della predisposizione di tali molluschi a prediligere riserve energetiche tissutali in forma di polisaccaridi piuttosto che di lipidi. La quota parte costituita da acidi grassi è per quasi il 75% rappresentata da acidi grassi insaturi, tre-quarti dei quali sono polinsaturi. Da un punto di vista strettamente nutrizionale gli acidi grassi della serie *n6* e della serie *n3* sono fra loro in rapporto sbilanciato a favore dei primi, in cui sono significativamente rappresentati l'acido linoleico e l'acido arachidonico. Ugualmente elevata è risultata la componente in sali minerali (più del doppio del normale contenuto in ceneri delle carni dei Vertebrati terrestri). Differenze interspecifiche sono state rilevate soprattutto a carico del contenuto in proteine e ceneri; il confronto intra-specifico fra prodotto d'allevamento e prodotto raccolto in natura non ha evidenziato sostanziali differenze. Le caratteristiche alimentari della *lumaca* si distinguono per un ridotto contenuto calorico conseguenza della limitata quantità di grasso di deposito nelle masse muscolari. L'apporto in acidi grassi di elevato valore nutrizionale da parte della massa muscolare del piede è ragguardevole.

Le lumache rispondono ai danni cutanei provocati dai predatori o dagli incidenti rigenerando le cellule danneggiate. Non sviluppano un'eccessiva reazione infiammatoria e inoltre le ferite sono riparate velocemente senza la formazione di cicatrici evidenti. I ricercatori hanno visto che la pelle delle lumache ha la stessa composizione di quella umana, con i medesimi elementi strutturali come il collagene e l'elastina. Quando la pelle del corpo umano è danneggiata o attaccata da microrganismi, la reazione infiammatoria che si scatena è molto differente e più forte di quella che si verifica nelle lumache. La lumaca reagisce ai danni cutanei producendo una grande quantità di muco che, attraverso la formazione di numerose bolle, bagna e aderisce completamente alla superficie della cute. Il fluido naturale idrata efficacemente la pelle e nello stesso tempo la protegge con i suoi peptidi antimicrobici, le sostanze antiossidanti e le molecole che incentivano l'ordinario processo rigenerativo che ristruttura e rinnova le cellule dei tessuti danneggiati.[2] La bava di lumaca ha peculiari caratteristiche adesive.per cui grazie a una particolare componente proteica, anche in concentrazioni minime, aderisce in maniera efficace, in ambienti umidi anche a superfici irregolari.[3] La bava di lumaca di *Cornu aspersum* (*Helix aspersa*) ha una composizione complessa di sostanze attive che la rendono un ingrediente cosmetico unico e non replicabile in laboratorio con un prodotto di sintesi o una miscela di essi. L'analisi

Correspondence: Francesca Grisafi, Dipartimento di Scienze Agrarie e Forestali, Università di Palermo, viale delle Scienze 11, 90127 Palermo, Italy.
E-mail: francesca.grisafi@unipa.it

Journal of Biological Research 2015; 88:5161

chimica quali-quantitativa ha evidenziato la presenza in particolare di allantoina 0.3-0.5%, collagene 0.1-0.3%, acido glicolico 0.05-0.1%, acido lattico 0.05-0.1%, anti-proteasi 1.3-1.8%, vitamine e minerali in tracce. In un recente lavoro[4] è stato messo in evidenza che il secreto di *Crymptophalus aspersa*, utilizzato per applicazioni cutanee, possiede proprietà rigenerative per la pelle umana per le quali sono state fatte interessanti ipotesi di meccanismo d'azione Studi clinici hanno dimostrato che prodotti cosmetici a base di secreto di *Cornu aspersum* (*Helix aspersa*) favoriscono la cicatrizzazione delle ustioni dei bambini riducendo la formazione del cheloide, l'iperpigmentazione e migliorando complessivamente l'aspetto estetico della cicatrice. In altri studi è stata confermata l'efficacia del secreto di *Cornu aspersum* (*Helix aspersa*) nella cicatrizzazione delle ustioni facciali di soggetti adulti. Sembra che la secrezione di *Cornu aspersum* (*Helix aspersa*) contribuisca con tutti i suoi componenti a promuovere la cicatrizzazione della ferita e la riduzione della formazione del cheloide. In particolare l'allantoina in essa contenuta ha spiccate proprietà cicatrizzanti già note anche per favorire la riparazione di ferite suppuranti, ulcere resistenti, emorroidi e varie infezioni dermatologiche. L'allantoina stimola la formazione tessutale e rende più rapida la cicatrizzazione delle ferite. Diverse preparazioni medicamentose topiche per la cicatrizzazione dei tessuti sono formulate con allantoina e si sono dimostrate particolarmente efficaci nel velocizzare la riparazione dei tessuti e nel ridurre la formazione delle cicatrici e dei cheloidi. L'acido glicolico e l'acido lattico, naturalmente contenuti nella bava di *Cornu aspersum* (*Helix aspersa*), contribuiscono a idratare e levigare la pelle riducendo l'iperpigmentazione e prevenendo la formazione delle smagliature anche durante la gravidanza. Migliorano l'estetica delle cicatrici anche di vecchia data. L'acido glicolico promuove il *turnover* epidermico e favorisce la proliferazione dei cheratinociti.

Bibliografia

1. Novelli E, Giaccone V, Balzan S, et al. Indagine sul valore dietetico-nutrizionale della lumaca. Confronto fra specie e fra soggetti raccolti in natura ed allevati. Ann. Fac. Medic. Vet. di Parma 2002;22:49-56.
2. Penazzi G. Potenziali proprietà ed usi in cosmesi del secreto di Helix aspersa o bava di lumaca. Cosmetic Technol 2010;13:25-8.
3. Pawlicki JM, Pease LB, Pierce CM, et al. The effect of molluscan glue proteins on gel mechanics. J Exp Biol 2004;207:1127-35.
4. Brieva A, Philips N, Tajedor R, et al. Molecular basis for the regenerative properties of a secretion of the mollusk Crymptophalus aspersa. Skin Pharmacol Physiol 2008;21:15-22.

High turnover rate of central histaminergic system in patients with Down syndrome and Alzheimer disease

M.C. Gueli

Department of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, Italy

It is well confirmed that a strong relationship exists between Down's syndrome (DS) and Alzheimer's disease (AD). Neurochemical investigations reported that many central neurotransmitter systems are similarly affected in aging Down and in Alzheimer patients, respectively. Airaksinem and colleagues[1] found numerous neurofibrillary tangles in the tuberomammillary area of the hypothalamus, where cell bodies of histaminergic neurons are located. While Mazurkiewicz-Kwilecki and colleagues[2] found deficits of the endogenous diamine, Cacabelos and colleagues[3] reported an increase of central histamine levels. In the present study, in order to test whether AD-like neuropathological changes involve the central histaminergic system, we measured the concentration of histamine, histidine as well as the activity of histidine decarboxylase (HDC) and histamine-N-methyltransferase (HMT) in temporal cortex (TC) of aging Down, Alzheimer and control patients.

Post-mortem samples (temporal cortex, TC; grey matter) of AD neuropathologically confirmed cases (72.1±7.6 years old), of karyotyped patients with DS (56.1±7.1 years old), and control adults (72,7±9.7 years old) were obtained from the MRC London Brain Bank for Neurodegenerative Diseases, Department of Neuropathology, Institute of psychiatry, London, U.K.

Each block of brain tissue from AD, DS and controls were thawed on ice and homogenized in ice-cold HDC-solution of 0.1 M sodium phosphate buffer (pH 6.8) containing dithiothreitol and antipain protease inhibitor. Homogenates of brain specimens were centrifuged at 12,000 x *g* for 20 min at 4°C. The supernatants were poured into CENTRIPEP-3 concentrators (Amicon), and centrifuged at 2,000 x *g* for two 10 min periods at 4°C. The clear extracts were stored in small quantities in Eppendorf tubes at -80°C until analysis. HDC activity has been measured with the procedure described by Gueli and colleagues[4] and briefly summarized. Extract aliquots were pre- incubated for 10 min with HDC assay-solution (0.1 M PBS, 0.2 mM DTT, 0.01 mM PLP, 0.1 mM Aminoguanidine), then incubation was started by adding 0.5 mM L-histidine for 0-3 h at 37°C. At the established times, the reactions were stopped with 60% ice-cold PCA, and stored overnight. Finally, the reaction mixture was centrifuged at 19,000 x *g* for 30 min at 4°C. The supernatants were withdrawn and filtered (0.45 m Millipore filter). The HPLC system consisted of a 600E Waters pump with a Waters 474 scanning fluorescence detector (ex 350 nm, em 450 nm). Chromatograms and calculations were performed by Empower TM2 Data Software. Histamine was separated and quantified after pre-column derivatization with Shore's o-phthalaldehyde reaction,[5] using a Spherisorb ODS2 analytical column, particle size 3 m (20 x 0.46 cm; Waters, Milano), a 10 μL injection volume, and a mobile phase of methanol, 20 mmol/L sodium acetate in water, acetic acid (55:43:2 v/v) and 0.33 mmol/L 1-octanesulfonic acid sodium salt. The flow rate was 1.0 ml/min. In order to measure HMT activity brain tissue was disperged with a glass Teflon homogenizer in 0.1 M PBS (pH 7.2). After centrifugation the supernatant was used for the radioenzymatic assay.[6] Histidine contents were measured using the procedure described by Borum.[7]

We observed a increase of histamine levels in temporal cortex of AD (+15%) patients. Down brains also showed a mild increase of the endogenous diamine concentration (+8%). HDC activity in both groups of diseased brains was significantly increased compared with controls (+59% for DS and +21% for AD, respectively). In accord to HDC actvity, HMT activity run in parallel in both pathological groups. In contrast to histamine, histidine levels were markedly decreased in temporal cortex of both pathological groups. These results put together leads us to think of a similar high turnover rate in the metabolic happenings of the histaminergic system in the temporal cortex of the patients with Alzheimer's disease and Down's syndrome. The fast histaminergic changes may contribute to the clinical manifestation of dementia in both disorders.

Correspondence: Department of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, via Del Vespro 129, 90127, Palermo, Italy.
E-mail: mariac.gueli@unipa.it

Journal of Biological Research 2015; 88:5161

References

1. Airaksinen MS, Paetau A, Paljarvi L, et al. Histamine neurons in human hypothalamus: anatomy in normal and Alzheimer diseased brains. Neuroscience 1991;9:465-81.
2. Mazurkiewicz-Kwilecki IM, Nsonwah S. Changes in the regional brain histamine and histidine levels in postmortem brains of Alzheimer patients. Can J Physiol Pharmacol 1989;67:75-8.
3. Cacabelos R, Yamatodani A, Niigawa H, et al. Brain histamine in Alzheimer's disease. Meth Find Exp Clin Pharmacol 1989;11:353-60.
4. Gueli MC. The mental disorders in the elderly: new therapeutic approaches. Rome: Brunello Ed.; 1997.
5. Shore PA, Burkhalter A, Cohn VH. A method for the fluorometric assay of histamine in tissues. J Pharmacol Exp Ther 1959;127:182-6.
6. Prast H, Gujrati V, Walser S, Philippu A. Histamine, histidine decarboxylase and histamine-N-methyltransferase in brain areas of spontaneously hypertensive rats, Naunyn-Schmiedeberg's Arch. Pharmacol 1988;338:573-83.
7. Borum PR. Manual for amino acid analysis of physiological samples. Atlanta, GA: American Association for Clinical Chemistry Publ.; 1997.

Novel mechanisms of phospholamban/SERCA2a modulation: phosphorylation *vs* S-nitrosylation and S-sulfydration

S. Imbrogno, A. Gattuso, R. Mazza, T. Angelone, M.C. Cerra

Department of Biology, Ecology and Hearth Sciences, University of Calabria, Arcavacata di Rende (CS), Italy

Phospholamban (PLN), a small protein closely associated with the cardiac sarcoplasmic reticulum (SR), has been identified and sequenced in many vertebrates, from fish to human. The very high homology among the sequence underlines the old evolutionary history of this protein, as well as its importance in myocytes dynamics. By modulating the intracellular calcium transient it represents the major determinant of cardiac contractility and relaxation. Alternate PLN phosphorylation/dephosphorylation determines SERCA2a on/off state, and thus the rate of SR refilling with Ca^{2+}, with a consequent impact on myocardial relaxation and contraction. In its dephosphorylated state, PLN inhibits Ca^{2+} sequestration by SERCA2a[1] and this induces more Ca^{2+} to be available for the contractile apparatus, thus decelerating relaxation; when PLN is phosphorylated this inhibition is relieved and Ca^{2+} is actively pumped into the SR causing an increased rate of myocardial relaxation.[2] A phosphorylation-dependent activation of PLN was observed by us both in mammalian (rat)[3-5] and non-mammalian (fish),[6] (amphibian)[7] vertebrate hearts. Recently, we have identified alternative, phosphorylation-independent, mechanisms of PLN/SERCA2a regulated Ca^{2+} reuptake, such as S-nitrosylation[4-6] and S-sulfhydration.[8] S-nitrosylation, the covalent modification of a protein cysteine thiol by a nitric oxide (NO) group to generate an S-nitrosothiol (SNO), is recognized to be important in regulating protein function.[9] This is particularly relevant in the heart, in which several proteins of critical significance were identified as potential targets for S-nitrosylation.[10] Using the biotin switch method[11] (Figure 1), we demonstrated that in the eel[6] and rat[4,5] heart PLN represents an important target for S-nitrosylation. In particular, in the eel it has been observed that the Frank-Starling response is modulated by a nitric oxide-dependent S-nitrosylation of PLN, which in turn improves myocardial relaxation.[6] In the rat heart, PLN S-nitrosylation appeared involved in the lusitropic action of several cardioactive substances, such as Catestatin and 17-β-estradiol.[4,5] A modified biotin switch method, using S-methyl methanethiosulfonate (MMTS) as an alkylating reagent, was recently used to identify a large number of proteins that may undergo S- sulfhydration.[12] Similarly to S-nitrosylation, S-sulfhydration represents an important signal which modulates many biological processes. In frog and rat heart, NaHS (a donor of H_2S) increases protein S-sulfhydration.[8] In the rat, Western Blotting of membrane fraction revealed two bands corresponding to the apparent molecular weights of PLN monomer (6kDa) and dimer (12 kDa) as putative targets for S-sulfhydration. This was confirmed by immunoprecipitation of the membrane protein fraction with anti-PLN antibody which revealed an increase of PLN S- sulfhydration in NaHS-treated hearts, particularly evident in the case of the 12kDa band[8] (Figure 2). Taken together, these results propose S-nytrosilation and S-sulfhydration of PLN as novel mechanisms for SERCA2a regulation which in turn modulates myocardial inotropic and lusitropic properties.

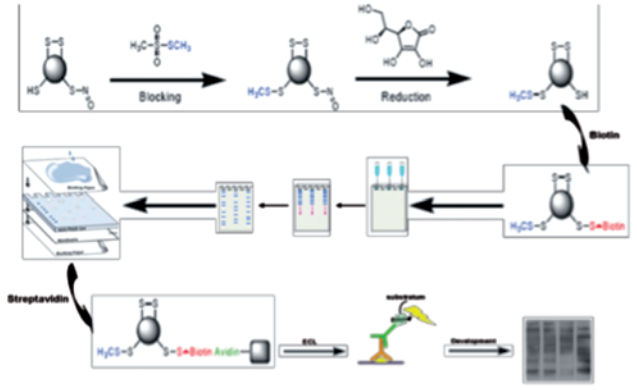

Figure 1. Biotin switch assay.

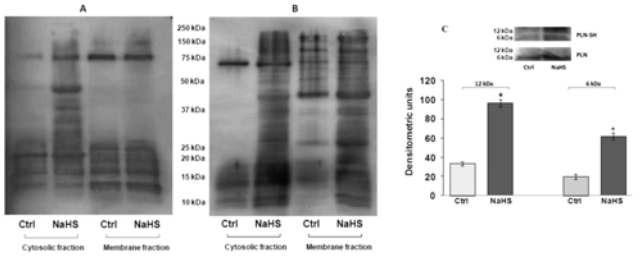

Figure 2. Cardiac protein S-sulfhydration in frog (A) and rat (B); C) Western blot analysis of immunoprecipitated PLN (rat).

Correspondence: Sandra Imbrogno, Department of Biology, Ecology and Hearth Sciences, University of Calabria, Ponte P. Bucci 6C, 84036 Arcavacata di Rende (CS), Italy.
E-mail: sandra.imbrogno@unical.it

Journal of Biological Research 2015; 88:5161

References

1. Kimura Y, Kurzydlowski K, Tada M, MacLennan DH. Phospholamban inhibitory function is activated by depolymerization. J Biol Chem 1997;272:15061-4.
2. Schmidt AG, Edes I, Kranias EG. Phospholamban: a promising therapeutic target in heart failure? Cardiovasc Drugs Ther 2001;15:387-96.
3. Angelone T, Quintieri AM, Brar BK, et al. The antihypertensive chromogranin a peptide catestatin acts as a novel endocrine/paracrine modulator of cardiac inotropism and lusitropism. Endocrinology 2008;149:4780-93.
4. Angelone T, Quintieri AM, Pasqua T, et al. Phosphodiesterase type-2 and NO-dependent S-nitrosylation mediate the cardioinhibition of the anti-hypertensive catestatin. Am J Physiol Heart Circ Physiol 2012;302:431-42.
5. Filice E, Angelone T, De Francesco EM, et al. Crucial role of phospholamban phosphorylation and S-nitrosylation in the negative lusitropism induced by 17β-estradiol in the male rat heart. Cell Physiol Biochem 2011;28:41-52.
6. Garofalo F, Parisella ML, Amelio D, et al. Phospholamban S-nitrosylation modulates starling response in fish heart. Proc Biol Sci 2009;276:4043-52.
7. Mazza R, Gattuso A, Mannarino C, et al. Catestatin (chromogranin A344-364) is a novel cardiosuppressive agent: inhibition of isoproterenol and endothelin signaling in the frog heart. Am J Physiol Heart Circ Physiol 2008;295:113-22.
8. Mazza R, Pasqua T, Cerra MC, et al. Akt/eNOS signaling and PLN S-sulfhydration are involved in H2S-dependent cardiac effects in frog and rat. Am J Physiol Regul Integr Comp Physiol 2013; 305:443-51.
9. Hess DT, Matsumoto A, Kim SO, et al. Protein S-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol 2005;6:150-66.
10. Lima B, Forrester MT, Hess DT, Stamler JS. S-nitrosylation in cardiovascular signaling. Circ Res 2010;106:633-46.
11. Jaffrey SR, Snyder SH. The biotin switch method for the detection of S-nitrosylated proteins. Sci STKE 2001:86:11.
12. Mustafa AK, Gadalla MM, Sen N, et al. H2S signals through protein S-Sulfhydration. Sci Signal 2009;2:72.

The osteogenic differentiation of bone chip-derived mesenchymal stem cells is controlled via specific receptor signaling

C. Kaebisch,[1] J.K. Gorski,[2] Y.A. Issa,[3] M.Winter,[4] E. Tobiasch[1]

[1]Department of Natural Sciences, University of Applied Sciences, Rheinbach, Germany; [2]Swanson School of Engineering, University of Pittsburgh, PA, USA; [3]Bethlehem University, Palestine; [4]Oral Surgery Practice, Rheinbach, Germany

Mesenchymal stem cells (MSCs) are an attractive cell source for Regenerative Dentistry in particular due to their ability to differentiate towards osteoblasts, among other lineages. Tooth and jaw bone loss are frequent sequelae of traumatic and pathological conditions in both the young and the elderly and must be met by appropriate prosthetic replacements. For successful osseointegration of the dental implant a sufficient bone level is necessary. Besides the utilization of bone autografts or synthetic biomaterials, medical research is more and more focused on the utilization of MSCs. Compared to cells obtained from liposuction material, ectomesenchymal stem cells derived from the head area *e.g.* out of dental follicles or particulate, non-vascularized bone chips show a higher differentiation potential towards osteoblasts. This implies that due to their different origin, ectomesenchymal stem cells are stronger committed towards hard tissues and are therefore interesting candidate cells for bone regeneration.[1,2]

Parathyroid hormone-related protein (PTHrP) is known to be involved in tooth eruption. It acts as a signaling molecule that stimulates local bone resorption.[3] Recently, PTHrP was found to affect the MSC differentiation process. The differential expression level of specific PTHrP isoforms might be considered as a molecular signature associated with the respective differentiation state during osteogenesis.[4] Moreover, we could show that in addition to the role of purinergic 2 (P2) receptors in cellular processes such as proliferation, migration and apoptosis, they are also involved in stem cell differentiation. Several P2 receptor subtypes play a role in key steps during osteogenic lineage commitment. Further development of MSCs into progenitor cells, pre-osteoblasts and osteoblasts seemed to be triggered via the alteration of their P2 receptor expression patterns.[5]

Human mesenchymal stem cells were isolated from bone chip material harvested during oral surgery intervention. Their stem cell character was demonstrated by plastic-adherence and expression of the surface markers CD73, CD90, and CD105 following differentiation along the osteogenic lineage.[6] The mineralization process was monitored by Alizarin Red S staining of extracellular matrix components. Among the examined P2 receptor subtypes, down-regulation of P2Y14 appeared to be involved in the onset of this differentiation process. Today several artificial P2 receptor ligands are present in the market. The administration of a stimulating P2Y14 receptor ligand had a direct influence on the osteogenic differentiation potential. More precisely, the application of the potent and selective P2Y14 agonist MRS 2690 led to a dose-dependent reduction of the extracellular mineralization.

Taken together, bone chip-derived mesenchymal stem cells are promising candidate cells for bone replacement. Here we show that they are capable of differentiating into osteoblasts. The application of an artificial receptor agonist confirmed the functional role of P2Y14 during osteogenesis. Therefore, it is of major interest to develop selective and potent antagonists directed against this recently discovered member of the purinergic receptor family. Controlling the P2Y14 receptor signaling might improve future bone tissue engineering approaches in regenerative dentistry.

Correspondence: Edda Tobiasch, Department of Natural Sciences, University of Applied Sciences, Bonn-Rhine-Sieg, Rheinbach, Germany.
E-mail: edda.tobiasch@h-brs.de

Journal of Biological Research 2015; 88:5161

References

1. Haddouti EM, Skroch M, Zippel N, et al. Human dental follicle precursor cells of wisdom teeth: isolation and differentiation towards osteoblasts for implants with and without scaffolds. Mat-Wiss Werkstofftech 2009;40:732-7.
2. Kaebisch C, Winter M, Khan D, et al. Examination of the quality of particulate bone chip material. (in press).
3. Philbrick WM, Dreyer BE, Nakchbandi IA, Karaplis AC. Parathyroid hormone-related protein is required for tooth eruption. P Natl Acad Sci USA 1998;95:11846-51.
4. Longo A, Librizzi M, Naselli F, et al. PTHrP in differentiating human mesenchymal stem cells: Transcript isoform expression, promoter methylation, and protein accumulation. Biochimie 2013;95:1888-96.
5. Zippel N, Limbach CA, Ratajski N, et al. Purinergic receptors influence the differentiation of human mesenchymal stem cells. Stem Cells Dev 2012;21:884-900.
6. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. Cytotherapy 2006; 8:315-7.

In vivo assay for the identification of potential inhibitors of epithelial-mesenchymal transition

R. La Tona,[1] A. Lavanco,[2] L. Anello,[2] A. Bongiovanni,[2] D.P. Romancino,[2] M. Di Bernardo[2]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, Palermo; [2]Institute of Biomedicine and Molecular Immunology, National Research Center, Palermo, Italy

We designed and developed a simple and reproducible screening assay to explore the ability of a number of small compounds, interfering with evolutionary conserved signalling pathways, to inhibit epithelial mesenchimal transition (EMT) *in vivo*. EMT is an important process during development by which fully polarized epithelial cells acquire mesenchymal, fibroblast-like properties and show reduced intercellular adhesion and increased motility. EMT mechanism is reactivated in tumor progression, tissue invasion, metastasis and the acquisition of resistance to therapy.[1,2] Targeting EMT, therefore, represents an important strategy for cancer treatment.

Sea urchin embryos provide a useful system to study EMT progression, just few hours after fertilization. The process is well characterized. Prior to ingression, the future primary mesenchyme cells (PMC) are indistinguishable from the neighbouring epithelial blastomeres, but their regulatory apparatus is actively preparing new molecular programs by which cells are going to turn on the expression of mesenchyme-specific molecular markers. Thus, at the right time PMCs, previously adherent to the adjacent epithelial cells via cadherin and adherens junctions, loose cell adhesion, enter the basal lamina and move through the blastocoele. Later, after migration to the proper *loci*, the specified PMCs will give rise to the embryonic skeleton.[3]

So far, potential EMT inhibitor compounds have been identified using a carcinoma cell line specifically induced to undergo EMT by the activation of growth factor signalling pathways.[4] Nevertheless, this approach restricts the research to bio-molecules only affecting the selected growth factor induced signals and in some cases the reporter cell lines are not responsive to all exogenous growth factors known to be EMT inducers.

Here, we propose an *in vivo* screening assay, using sea urchin embryos (Figure 1); we picked out a selection of pharmacologically active compounds from a commercial library (LOPAC[1280]TM, Sigma-Aldrich) and tested their ability to inhibit EMT in embryos. These molecules are known to interfere with some evolutionarily conserved signalling pathways: P38 mitogen-activated protein kinase (MAPK), platelet-derived growth factor receptors (PDGF-R) epidermal growth

factor receptor (EGFR) tyrosine-protein kinase (Src), Glycogen synthase kinase 3 (GSK-3). We set up the experiments as follows: two different batches of *Paracentrotus lividus* embryos at zygote (just post-fertilization) or hatching blastula (12h post-fertilization and 4h prior PMC ingression) stages were incubated in multiwell plates in the presence of different concentrations of the selected drug. Treated or control embryos were then monitored under an inverted microscope and scored for timely precise PMC formation, number and migration capability. Embryos were then photographed, phenotipically classified and in some experiments assayed for the expression of specific antigens. We obtained evidence that some of these compounds inhibit EMT and prevent the expression of mesenchymal specific molecular markers. We propose this low-, medium-throughput Sea Urchin embryonic EMT Assay (SU-EMTA) as an affordable and useful method to screen a high number of compounds, with potential anti-metastatic activity.

Correspondence: Daniele Romancino, Institute of Biomedicine and Molecular Immunology, National Research Center, via Ugo La Malfa 153, 90146 Palermo, Italy.
E-mail: daniele.romancino@ibim.cnr.it

Fundung: the present study has been partially supported by a grant from the Italian Ministry of Economy and Finance to the CNR for the Project *FaReBio di Qualità*.

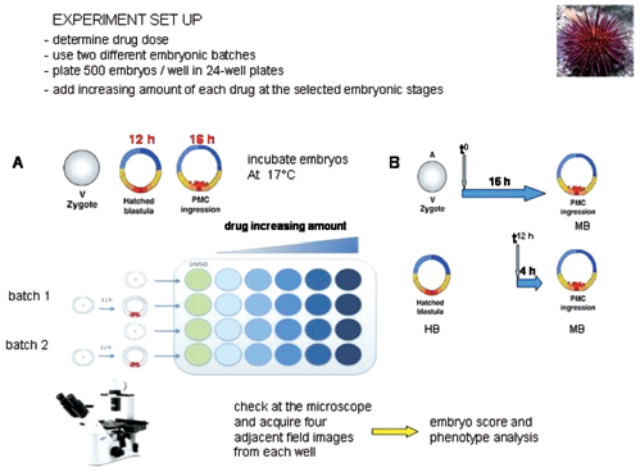

Figure 1. A) Set up of a standard epithelial mesenchimal transition assay on sea urchin embryos; B) drug addition at fertilization (zygote) for 16 h or at hatching blastula stage (HB) for 4 h. Phenotype analysis at mesenchyme blastula stage (MB) and later (36 h after fertilization).

References

1. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009;119:1420-8.
2. Lim J, Thiery JP. Epithelial-mesenchymal transitions: insights from development. Development 2012;139:3471-86.
3. Wu S-Y, Ferkowicz M, McClay DR. Ingression of primary mesenchyme cells of the sea urchin embryo: a precisely timed epithelial mesenchymal transition. Birth Defects Research 2007; 81:241-52.
4. Chua KN, Sim W-J, Racine V, et al. A cell-based small molecule screening method for identifying inhibitors of epithelial-mesenchymal transition in carcinoma. PLoS ONE 2012;7:e33183.

Premature termination codon 124 derivatives as a novel approach to improve the read-through of premature amber and ochre stop codons

L. Lentini, R. Melfi, I. Pibiri, A. Pace, A. Di Leonardo

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

Nucleotide changes within an exon may alter the trinucleotide normally encoding a particular amino acid, such that a new *stop* signal is transcribed into the mRNA open reading frame. This causes the ribosome to prematurely terminate its reading of the mRNA, leading to the lack of production of a normal full-length protein. Such premature termination codon (PTC) mutations occur in an estimated 10 to 15% of many genetically based disorders.[1]

Pathological nonsense mutations resulting in TAG (40.4%), TGA (38.5%), and TAA (21.1%) occur in different proportions to naturally occurring stop codons.[2] Several genetic disorders are characterized by *opal* (TGA; Cystic fibrosis, Duchenne/Becker muscular dystrophy), *amber* (TAG; b-thalassemia, emphysema, cystic fibrosis) and *ochre* mutations (TAA; APC gastric cancer, Haemophilia B, Hypothyroidism).[3] Messenger RNA containing a nonsense mutation is often degraded rapidly through the process of nonsense-mutation-mediated decay (NMD) resulting in the lack of the protein.[4]

A recent approach to directly overcome the deleterious effects caused by nonsense mutations is

represented by readthrough strategies which take advantage of the known properties of aminoglycosides that can suppress stop codons.[5] Several aminoglycosides (gentamicin, amikacin, hygromycin, *etc*.) can suppress the accurate identification of translation termination codons in cultured eukaryotic cells. Unfortunately, aminoglycoside action lacks specificity resulting in readthrough of many correctly positioned stop codons. Consequently, long-term use of aminoglycosides may originate toxic aggregates or dominant negative readthrough products.[6]

By an high throughput screening it was identified the PTC124 (Ataluren), a small molecule that has been suggested to allow PTCs readthrough.[7] However, despite the results obtained on opal mutation it was shown that it has a lower activity against *ochre* and *amber* nonsense mutations.[7]

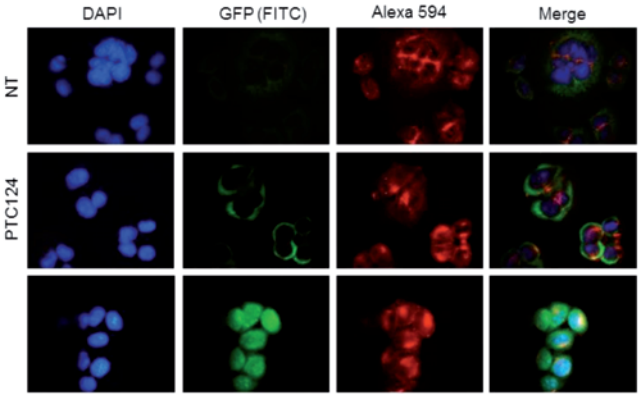

Figure 1. Immunofluorescence analysis of the H2B-GFP protein in H2B-GFP-*amber* stably transfected HeLa cells after 24 hours exposition to PTC124 and N.1 derivative.

In the attempt to identify molecules with an activity against *ochre* and *amber* nonsense mutations, we designed and synthesized new PTC124 derivatives to be tested in human cultured cells to see if they have higher and wider activity towards PTCs than PTC124. To this aim we generated a reporter vector with non-sense mutation by introducing in the pBOS-H2BGFP plasmid a TAG codon (*amber*) and TAA codon (*ochre*) by site-directed mutagenesis. PCR and sequencing analyses confirmed the presence of the stop codons in the plasmids that were transfected in HeLa cells to explore the ability of the derivatives to promote the translational read-through. Immunofluorescence analyses showed that one of the analyzed derivatives was able to restore GFP fluorescence in HeLa H2BGFP-*amber* cells, as indicated by GFP-localization in the nuclei of treated cells (Figure 1). This positive response also confirmed the correct functioning of the model system which will allow us to perform the screening of a greater number of molecules with read-through action.

Correspondence: Laura Lentini, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze 16, 90128, Palermo, Italy.
E-mail: laura lentini@unipa.it

Funding: this work was funded by the Italian Cystic Fibrosis Research Foundation, (Delegazione di Lecce, Delegazione di Vittoria, Ragusa).

References

1. Finkel RS. Read-through strategies for suppression of nonsense mutations in Duchenne/Becker muscular dystrophy: aminoglycosides and ataluren (PTC124). J Child Neurol 2010;25:1158-64.
2. Bidou L, Allamand V, Rousset JP, Namy O. Sense from nonsense:

therapies for premature stop codon diseases. Trends Mol Med 2012;18:679-88.
3. Mort M, Ivanov D, Cooper DN, Chuzhanova NA. A meta-analysis of nonsense mutations causing human genetic disease. Hum Mutat 2008;29:1037-47.
4. Mendell JT, Dietz HC. When the message goes awry: disease-producing mutations that influence mRNA content and performance. Cell 2001;107:411-4.
5. Hermann T. Aminoglycoside antibiotics: old drugs and new therapeutic approaches. Cell Mol Life Sci 2007;64:1841-52.
6. Diop D, Chauvin C, Jean-Jean O. Aminoglycosides and other factors promoting stop codon readthrough in human cells. C R Biol 2007;330:71-9.
7. Welch EM. PTC124 targets genetic disorders caused by nonsense mutations. Nature 2007;447:87-91.

New more polar symmetrical choline kinase inhibitors II: study of setting up a new scaffold for the cancer therapy

L.C. López-Cara,[1] F. Castro-Navas,[1] S. Aiello,[2] M.A. Gallo-Mezo,[1] A. Espinosa,[1] A. Entrena-Guadix[1]

[1]Department of Pharmaceutical and Organic Chemistry, University of Granada, Spain; [2]Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, Italy

Research into the anti-tumour properties of biscationic compounds has received significant attention over the last few years. In the challenge to improve modern cancer chemotherapy, the search of new drugs with higher therapeutic index and lower capacity to induce resistance is an active field of investigation in medicinal chemistry. As part of our drug research program in searching modified biscationic compounds that show strong growth inhibitory activities against a two cancer cell lines,[1-3] we were interested in more polar biscationic compounds derivatives, which should constitute an important class of new compounds for their potential pharmaceutical applications.

A novel family of 1,1'-[biphenyl-4,4'-diyldi(methylene)]dipyridinium salts containing a pair of pyridines as linker of the framework of the biscationic compounds, like hypothetical hydrogen bond acceptors with the enzyme choline kinase, were synthesized and they are being evaluated as inhibitors of choline kinase. Their antiproliferative activity will be evaluated in the future as well.

References

1. Gómez-Pérez V. Novel 4-Amino bis-pyridinium and bis-quinolinium derivatives as choline kinase inhibitors with antiproliferative activity against the human breast cancer SKBR-3 cell line. Chem Med Chem 2012;7:663-9.
2. Sánchez-Martín RM. Symmetrical bis-quinolinium compounds: new human choline kinase inhibitors with antiproliferative activity against the HT-29 cell line. J Med Chem 2005;48:3354-63.
3. Martín-Cantalejo Y. Synthesis and Biological activity of new bispyridinium salts of 4,4'-bispyridyl-5,5'-perfluoroalkyl-2,2'-bioxazoles. Eur J Med Chem 2011;50:5562-7.

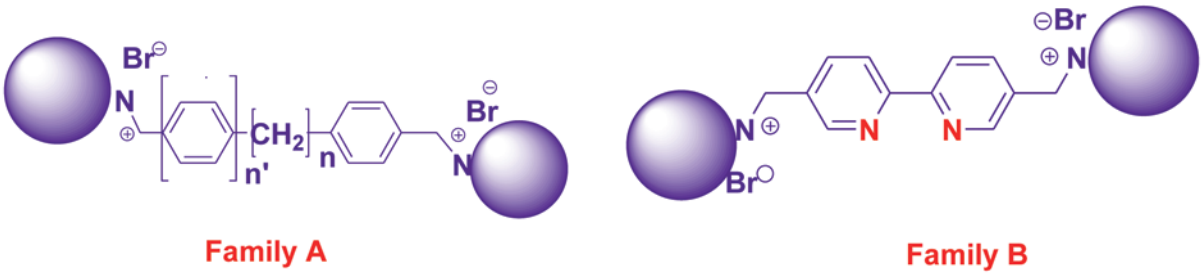

Figure 1. General structures of symmetrical biscationic inhibitors of choline kinase Family A was previously published.[1-3] Family B is described in this work.

Correspondence: Luisa Carlota López-Cara, Department of Pharmaceutical and Organic Chemistry, University of Granada, Campus cartuja, 18071 Granada, Spain.
E-mail: lcarlotalopez@ugr.es

Journal of Biological Research 2015; 88:5161

Two proton pumps are required for regulation of bicarbonate secretion in the intestine of *Sparus aurata*

M. Lupica Infirri,[1] E.S.M. Carvalho,[2] F. Trischitta,[1] J. Fuentes[2]

[1]Department of Biological and Environmental Sciences, University of Messina, Italy; [2]Center of Marine Sciences, University of Algarve, Faro, Portugal

Acid and base transport through cell membranes is essential for all cells in order to maintain acid-base balance. In some cells this transport is used for peculiar purposes. Two examples are the pancreatic duct cells and the enterocytes of marine teleosts. The former secrete a HCO_3^- -rich fluid necessary to increase the chime pH and to activate the intestinal enzymes. Marine teleost enterocytes actively secrete HCO_3^- in order to precipitate the large amounts of Ca^{2+}(and Mg^{2+}) introduced by swallowing seawater. Divalent cation precipitation has the role to reduce the osmotic pressure of the intestinal fluid, thus allowing water absorption, necessary to compensate water loss into the hyperosmotic external medium. In addition it inhibits Ca^{2+} overabsorption and may prevent renal stones formation.[1,2]

The mechanisms of base secretion and their regulation in seawater teleosts, including *Sparus aurata*, have been extensively studied.[3-6] In this paper the involvement of proton pumps in bicarbonate secretion (BCS), already suggested in mammal pancreatic duct cells,[7] was investigated in isolated tissues mounted in Ussing chambers, where the transepithelial electrical parameters (short circuit current, Isc, and tissue resistance, R_t) were also measured. We found that the apical addition of 10 μM omeprazole, a specific H^+-K^+-ATPase inhibitor produced a significant decrease of BCS and a parallel increase in I_{sc} positivity in both the anterior intestine and the rectum, irrespective of the presence of serosal bicarbonate. Experiments performed with the aim of testing the reversibility of omeprazole effect on BCS led to an intriguing result: a large and transient increase of BCS following omeprazole removal from the apical solution. This response was strongly reduced in tissues pre-incubated with 100 μM colchicine, a known inhibitor of microtubule polymerization.

Acetazolamide, a carbonic anhydrase inhibitor, which strongly inhibited BSC, was not able to modify Isc in the anterior intestine but produced a small increase in I_{sc} positivity in the rectum. Apical addition of the selective inhibitor of the V-type H^+-ATPase, Bafilomycin A1 (0.1 μM), was not able to reduce BCS in the rectum while exhibited a significant inhibitory effect in the anterior intestine. The addition of omeprazole after Bafilomycin A1 produced a further BCS inhibition. In contrast Bafilomycin A1 was ineffective when tested in tissues in which omeprazole had produced its maximal inhibition of BCS.

The results of our experiments lead us to assume that two proton pumps are necessary for the regulation of bicarbonate secretion in the intestine of *Sparus aurata*.

Omeprazole experiments suggest that H^+-K^+-ATPase plays a role in BCS both in the anterior intestine and in the rectum, this role seems independent from the source of secreted bicarbonate (endogenously formed or extracellular). The possibility that omeprazole can inhibit carbonic anhydrase[8] and hence the intracellular bicarbonate generation, seems ruled out by the observation that acetazolamide, that inhibited BCS, produced effects on Isc that are different from those produced by omeprazole both in the anterior intestine and in the rectum. However an effect of omeprazole on Cl^- channel[9] cannot be excluded.

The finding that the large and transient increase of BCS, observed when omeprazole was removed from the luminal solution, was reduced in tissues pre-incubated with colchicine could suggest that omeprazole removal stimulates the H^+-K^+-ATPase activity, due to an insertion of proton pumps into plasma membrane by a mechanism microtubule dependent, already demonstrated in the gastric parietal cells.[10]

Bafilomycin experiments suggest that the V-type H^+-ATPase is also involved in the regulation of bicarbonate secretion in the anterior intestine but has a minor role in the rectum. However further studies are necessary to confirm our conclusions.

The sensitivity of intestinal BCS to omeprazole and bafilomycin points the functional involvement of two proton pumps in the simultaneous secretion of H^+ and HCO_3^- in the intestine that could be explained by two hypotheses: i) the titration of luminal HCO_3^- with protons near the luminal surface of the enterocyte could reduce the net HCO_3^- gradient across the luminal membrane to sustain the action of a Cl^-/ HCO_3^- exchanger; ii) apical H^+ secretion could defend cytosolic pH in the apical region where carbonic anhydrase is abundant and catalyzes the hydration of CO_2 to form H^+ and HCO_3^-, necessary for Cl^-/ HCO_3^- exchange. Proton removal could prevent reversal of the hydration reaction.[3]

Correspondence: Francesca Trischitta, Department of Biological and Environmental Sciences, University of Messina, viale F. Stagno d'Alcontres 31, 98166 Messina, Italy.
E-mail: ftrischi@unime.it

Journal of Biological Research 2015; 88:5161

References

1. Wilson RW, Wilson JM, Grosell M. Intestinal bicarbonate secretion by marine teleost fish - why and how? Biochim Biophys Acta 2002; 1566:182-93.
2. Wilson J, Grosell M. Intestinal bicarbonate secretion in marine teleost fish-source of bicarbonate, pH sensitivity, and consequences for whole animal acid-base and calcium homeostasis. Biochim Biophys Acta 2003;1618:163-74.
3. Grosell M, Mager EM,Williams C, Taylor JR. High rates of HCO_3^-

secretion and Cl-absorption against adverse gradiewnts in the teleost intestine: the involvement of an electrogenic anion exchanger and H^+-pump metabolon? J Exp Biol 2009;212: 1684-96.
4. Fuentes J, Power DM, Canario AVM. Parathyroid hormone-related protein- stanniocalcin antagonism in regulation of bicarbonate secretion and calcium precipitation in a marine fish intestine. Am J Physiol-Reg Int Comp Phys 2010;299:150-8.
5. Faggio C, Torre A, Lando G, et al. Carbonate precipitates and bicarbonate secretion in the intestine of sea bass, Dicentrarchus labrax. J Comp Physiol B 2011;181:517-25.
6. Ferlazzo A, Carvalho ESM, Gregorio SF, et al. Prolactin regulates luminal bicarbonate secretion in the intestine of the sea bream (Sparus aurata L.). J Exp Biol 2012;215:3836-44.
7. Novak I, Wang J, Henriksen KL, et al. Pancreatic bicarbonate secretion involves two proton pumps. J Biol Chem 2011;286:280-9.
8. Puscas I, Coltau M, Baican M, Domuta G. Omeprazole has a dual mechanism of action: it inhibits both H^+ K^+ ATPase and gastric mucosa carbonic anhydrase enzyme in humans (in vitro and in vivo experiments). J Pharm Exp Ther 1999;290:530-4.
9. Schmarda A, Dinkhauser P, Gschwentner M, et al. The gastric H,K-ATPase blocker lansoprazole is an inhibitor of chloride channels. Brit J Pharmacol 2000;129:598-604.
10. Yao X, Forte JG. Cell biology of acid secretion by parietal cell. Ann Rev Physiol 2003;65:103-31.

Ecology and energy transfer from planktonic organisms to small pelagic fishes in the North West Mediterranean Sea

E. Macaluso,[1] M.P. Olivar,[2] F. Trischitta,[1] I. Palomera[2]

[1]Department of Biological and Environmental Sciences, University of Messina, Italy; [2]Institute of Marine Sciences, Barcelona, Spain

Ecosystem ecology is today one of the most important scientific tools that have direct applications for management and conservation of marine enviroment. I had possibility to work as an Erasmus Placement student at Barcelona in the ICM-CSIC (Institute of Marine Sciences) in a project named ECOTRANS leadered by Dr. Isabel Palomera. The project will last for three years (2012-2014). It's a scientific project funded by the Spanish Ministry of Economy and Competitivness and the team of researchers which I was part of, are studying the trophic relations between different aspects of the pelagic ecosystem in the Western Mediterranean Sea, with a special focus on the dynamics and ecological role of small pelagic fish,in particular European anchovy (*Engraulis encrasicolus*), European sardine (*Sardina pilchardus*), and sardinella (*Sardinella aurita*) such as dominant and key species in terms of biomass and production, and their transfer of energy as trophic levels among the trophic chain. The area of interest and study includes the continental shelf in front of the Ebro river delta from Tarragona northwards until Castellón de la Plana. The total study area is about 1800 squared miles (Figure 1). This project is articulated into two fundamental, parts, necessary to recollect all the informations and datas,the big one is the sampling by two oceanographic-fishing cruises, that will be conducted to cover the following objectives: i) sampling micro and mesozooplankton; ii) sampling larvae of selected pelagic; and iii) trawl sampling (pelagic and benthonic fish) to get the species and functional groups biomass in the ecosystem that will allow to actualize the existing trophic models. I participate at the second cruise that was conducted during the spawning of anchovy as one of the objectives was to get their larvae, and also larva of gilt sardine, horse-mackerel and mackerel. The plankton methods conducted at wich I mostly collaborate were the sampling with plankton nets aimed at obtaining the microplankton and mesozooplankton biomass, and the fish larvae. We used various kind of nets everyone with a different technique of sampling, depending if we need to catch microplancton or mesozooplancton, like CALVET net and WP2; also Bongo net sampling for catching fish larvae in the same station and finally samples with RMT-1m network to capture larvae and crustaceans in depth. There will be also catches with PATIN neuston for capturing larger larvae, which are in the surface layers during the night. The plankton samples collected for the study of micro and mesozooplankton are being used to calculate the biomass (dry weight) of each group and another part for the corresponding biochemical analysis (calories, fat and stable isotopes) of the functional groups of plankton and the larvae. At same time the larvae samples recollected will be also conserved in cryovials at -80°C. The rest of each fish larvae will be conserved in 5% formalin for subsequent separation and determination of the size structure. Another important part of this study was developed consequently at the laboratory to follow the protocols for plankton preparation and analysis divided into: i) calculate and obtain the data of biomass abundance and distribution of micro and mesoplankton; ii) sorting functional group of zooplankton as Copepods, larvae of Decapods, Eufasiacea and Mysidiacea, Cladocers and Appendicularians that will be prepared for isotopic and calorimetric analysis; iii) identification and sorting of eggs and larvae of sardine and anchovy for each station. From all these data recollected we are attending a partial spatial-distribution on the area of study, like the use of SURFER software for plankton distribution analysis and know approximately the percentage of energy/biomass from intermediate trophic levels available to upper trophic levels.

Correspondence: Francesca Trischitta, Department of Biological and Environmental Sciences, University of Messina, viale F. Stagno d'Alcontres 31, 98166 Messina, Italy.
E-mail: ftrischi@unime.it

Journal of Biological Research 2015; 88:5161

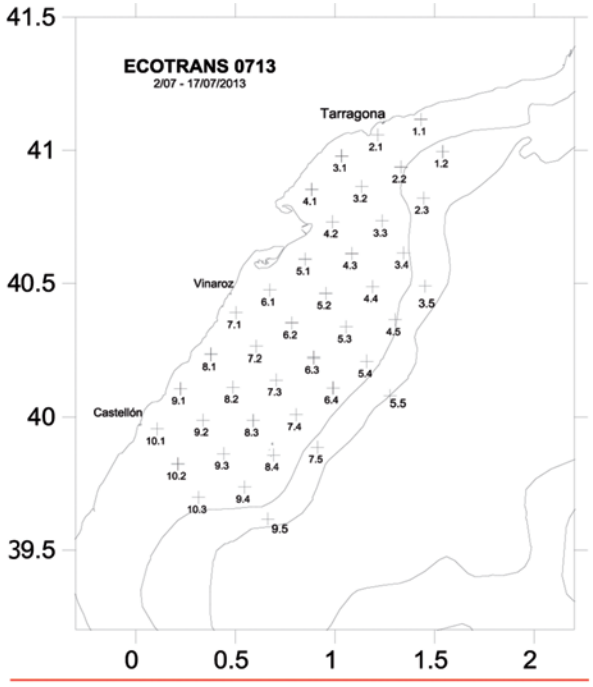

Figure 1. Sampling map of the study area indicating the grid of planktonic station.

INNOVAQUA project *Technological innovation for the improvement of productivity and competitiveness of Sicilian aquaculture*: aspects of experimental biology

A. Manganaro,[1] M. Sanfilippo,[1] C. Faggio,[1] G. Genovese,[1] C. Gugliandolo,[1] T.L. Maugeri,[1] M. Morabito,[1] F. Trischitta,[1] A. Santulli[2]

[1]Department of Biological and Environmental Sciences, University of Messina; [2]Department of Earth and Marine Sciences, University of Palermo, Italy

The INNOVAQUA project entitled *Technological Innovation for the improvement of productivity and competitiveness of Sicilian aquaculture* aims to stimulate the potential development of existing in Southern Italy aquaculture, through the definition of new technologies for the industry that require intensive researches and development in-house, and involving different stages of the production chain. The project holds in high regard the needs of health, safety and sustainability of consumers. The scientific partners of INNOVAQUA are: Acqua Azzurra SPA; University of Messina; University of Palermo; IAMC–CNR; IZS Sicily. The project has been founded by the Operative National Program Research and Competitiveness (PON R&C) to the Technological District *Agro Bio Pesca Ecocompatibile* of Sicily.

In order to maximize profitability and expansion in the increasingly competitive market, the project includes three specific work packages (WP 1-3) (Figure 1).

First, the activities planned in WP1 (Fish species diversification) aim to identify new fish species (umbra and amberjack) of commercial interest on which will be carried out tests of reproduction and larval rearing. A stock of breeding amberjack will be set up to develop suitable techniques for maintaining in captivity.

Second, the WP2 (Productivity increase of farmed fish) activities are designed to improve the productivity of seabass and seabream, representing almost the entire Mediterranean and European production. These activities represent an opportunity for industry development through importa nt technical-scientific progress and commercial applications.

Third, the WP3 (Actions to support innovation and competitiveness) activities will focus on the improvement of fish farming conditions, through the use of active molecules extracted from marine algae. The following activities (A1, A2, A3 and A4) of Experimental Biology will be developed (Figure 2).

A1. The algal biomolecules are of considerable interest in different sectors characterized by a wide spectrum of antibiotic activity and immunostimulant, as pharmaceuticals. Such molecules can greatly differ among related species and also within the same species. For this reason, the taxonomic identification of the species is carried out by the DNA barcoding techniques. The results will yield a list of local species producing bioactive macromolecules and protocols for the extraction of phyto-derivates. Local species of macroalgae producing bioactive macromolecules have been identified and obtained crude extracts will be characterized. Different methods of extraction on the basis of various active ingredients will also be tested.

A2. Diseases of bacterial origin are cause of considerable economic losses in aquaculture. The discovery of new bioactive molecules pro-

Figure 1. Three specific work packages included in the INNOVAQUA project.

Correspondence: Antonio Manganaro, Department of Biological and Environmental Sciences, University of Messina, viale F. Stagno d'Alcontres 31, 98166 Messina, Italy.
E-mail: amanganaro@unime.it

Funding: with the contribution of the project Innovaqua PON02_00451_3362185/1.

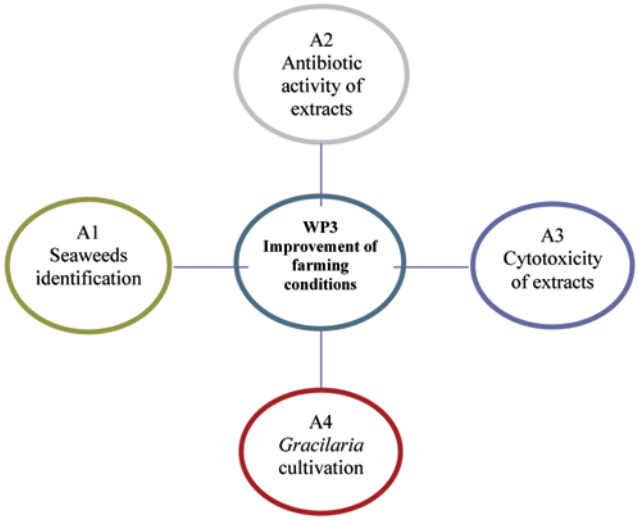

Figure 2. A1 to A4 activities of experimental biology developed.

duced by marine organisms is still today a promising challenge in the field of biotechnology, mainly in pharmaceuticals. The increasing phenomenon of resistance of pathogenic bacteria of humans and animals to current antibiotics raises the need for discovering novel active biomolecules useful in human therapy, in veterinary medicine and in the aquaculture industry, and with not any side effects on human and environmental health. Since the compounds extracted from marine algae have already been reported to have antibacterial and immunostimulant activities, in this study we analyze the extracts of algae belonging to the genera most widely distributed along our coasts against bacteria of great significance for fish and human health. Preliminary results indicate that the studied algal extracts could be sources of novel antibacterial compounds with potential use in the prevention and treatment of diseases of farmed fish.

A3. In order to evaluate cytotoxicity of algal extracts the common cytotoxicity test will be performed: the trypan blue assay that measures cytotoxicity based on alterations in plasma membrane permeability and consequent dye uptake, normally excluded by viable cells and the hemolysis assay, a sensitive and accurate tool used as a guide to assess the safety and utility of a molecule or pharmaceutical preparations. If the algal extracts will be neither cytotoxic nor hemolytic they will be administered to fish and afterwards studies on fish hematological parameters and on the gastric and intestinal transepithelial parameters, by short circuit current, I_{sc}, a measure of transepithelial ion transport will be carried out.

A4. Algae of the genus *Gracilaria* have been studied to verify various types of cultivation (on nets, on ropes). Once tested the growth of seaweeds in natural environment, a protocol for culturing these algae in the wastewater of a system of intensive aquaculture is in course of development, thus verifying the ability of seaweed to reduce the pollution load of wastewater. Also, the extractability of phycocolloids and agar from *Gracilaria* will be evaluated. The goal is the selection of strains of *Gracilaria* able to provide products of industrial interest and with capacity of phytoremediation of breeding water.

The experimental activity described is preparatory to evaluate the efficacy *in vivo* in experimental aquaculture plant. In fact, the antimicrobials and immune-boosting obtained from selected algal cultures will be tested on teleosts to assess their potential use as therapeutic and prophylactic agents.

Brief maternal separation procedures occurring early in life affect learning and memory in adult Wistar rats: sex-related differences in cognitive behaviour

R.A.M. Marino, F. Plescia, A. Brancato, G. Gambino, C. Cannizzaro

Department of Sciences for Health Promotion and Mother and Child Care Giuseppe D'Alessandro, University of Palermo, Italy

Introduction

Adverse life events during the neonatal period result in long-term effects on physiology and behavior.[1] Early postnatal experiences, such as a modification of the mother–infant interaction, may influence the development of neural systems that underlie the expression of neuroendocrine and behavioural responses to environmental challenges, involving changes in the hypothalamic-pituitary-adrenal (HPA) axis[2] together with decreased levels of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF).[3] Disturbances in mother-infant interaction represent a natural stressor which may lead to maladaptive development;[4] indeed protracted Maternal Separation (MS) reduces maternal care thus induce in abnormal HPA axis responses, hippocampal BDNF down-regulation[5] impaired memory formation.[6] The opposite is observed when maternal care is increased by a daily brief, maternal separation. The polymorphism of neuroendocrine processes and behavioural responses following brief MS procedure 7 includes gender-related differences; however very few studies exist on sex-specific behaviours.[7] Based upon these findings, the present study was carried out to investigate the consequences of a brief, maternal separation on declarative and spatial memory, focusing on sex related alteration due to the discrete effects that hormones may play on the brain circuits.

Materials and Methods

24 adult male and female Wistar rats were divided in the following experimental groups: maternally separated males (MS-m) and females (MS-f), and non-separated males (NS-m) and females (NS-f). Maternal manipulation involved a 15- minutes daily separation of litters from the mothers from postnatal day 2 (PND 2) to 21 (PND 21). Non separated rats were left undisturbed in their home cages until weaning. To assess the influence of maternal separation on cognitive function we used respectively: the Object Recognition (OR) Test for declarative memory and the Morris Water Maze (MWM) for spatial learning and reference memory, performing respectively place learning and probe sessions.

Results

Our results indicate that a brief, daily maternal separation was able to induce in MS-f group an increase in the time spent exploring the novel object in the OR in both after 1h ($P<0.001$), and

24h ($P<0.05$) retention intervals compared to NS-f controls, while no significant differences were observed in MS-m group when compared to respective controls.

In the MWM, during the Place learning paradigm (day 1) MS-m rats showed a reduction in escape latency ($P<0.001$) and, during the probe phase, an increase in time spent in the target quadrant after platform removal ($P<0.05$), compared to NS-m group. On the contrary, MS-f group showed non-significant differences in escape latency in the place learning compared to NS-f controls. MS-f rats spent more time ($P<0.05$) in the target quadrant in probe phase, compared to respective controls.

Conclusions

The present study was designed to examine the effects of brief daily mother–offspring separation on learning and memory performance, focusing on sex-related differences in declarative and spatial memory. In detail, our study showed that exposure to a brief, maternal separation results in sexually-dimorphic cognitive alterations that depend on the nature of the behavioral task: Iindeed we found that MS-f group outperformed MS-m group on Object Recognition Test, a working, non-spatial memory dependent task that utilizes both cortical and hippocampal input.[8] This result could be due to a potential improvement of the perirhinal cortex and dorsal hippocampus activity in females when compared to MS-m group and to controls.[9] In addition, in the Morris Water Maze, maternal separation affected learning in adult male and female rats in a task-specific manner. In particular, it was observed that this procedure enhanced the ability of processing spatial information only in male rats during the first day of place learning

Correspondence: Carla Cannizzaro, Department of Sciences for Health Promotion and Mother and Child Care Giuseppe D'Alessandro, University of Palermo, via del Vespro 129, 90127 Palermo, Italy.
E-mail: carla.cannizzaro@unipa.it

whereas, both groups showed improved performances during probe, confirming the discrete effects of maternal separation on mechanisms of memory storage. In conclusion, a brief, maternal separation affects learning and memory in adult rats not only in a sex-related, manner, but also in a task- specific way.[10] This study provides us with different outcomes useful to clarify how early life events can influence the behavioural adaptive mechanisms in adulthood in a sex specific manner probably due to differences in the modulation of hippocampal function and HPA axis response[11,12] highliting, overall, the main role played by a high mother-infant relationship in the correct development of physiology and behaviour in adulthood.

References

1. Gareau MG, Jury J, Perdue MH. Neonatal maternal separation of rat pups results in abnormal cholinergic regulation of epithelial permeability. Am J Physiol Gastrointest Liver Physiol 2007;293:198-203.
2. Mesquita AR, Pego JM, Summaveille T, et al. Neurodevelopment-milestone abnormalities in rats exposed to stress in early life. Neuroscience 2007;147:1022-33.
3. Griesbach GS, Hovda DA, Molteni R, et al. Voluntary exercise following traumatic brain injury: Brain-derived neurotrophic factor upregulation and recovery of function. Neuroscience 2004;125:129-39.
4. Daniels WMU, Pietersen CY, Carstens ME, Stein DJ. Maternal separation in rats leads to anxiety-like behavior and a blunted ACTH response and altered neurotransmitter levels in response to a subsequent stressor. Metab Brain Dis 2004;19:1-2.
5. Marais L, van Rensburg SJ, van Zyl JM, et al. Maternal separation of rat pups increases the risk of developing depressive-like behavior after subsequent chronic stress by altering corticosterone and neurotrophin levels in the hippocampus. Neurosci Res 2008; 61:106-12.
6. Knuth ED, Etgen AM. Long-term behavioral consequences of brief, repeated neonatal isolation. Brain Res 2007;1128:139-47.
7. Korosi A, Naninck EF, Oomen CA, et al. Early-life stress mediated modulation of adult neurogenesis and behavior. Behav Brain Res 2012;227:400-9.
8. Steckler T, Drinkenburg WHIM, Sahgal A, Aggleton JP. Recognition memory in rats. Concepts and classification. Prog Neurobiol 1998; 54:289-311.
9. Baxter MG. I've seen it all before: explaining age-related impairments in object recognition. Theoretical comment on Burke et al. Behav Neurosci 2010;124:706-9.
10. Noschang C, Krolow R, Mar Arcego D, et al. Neonatal handling affects learning, reversal learning and antioxidant enzymes activities in a sex-specific manner in rats. Int J Dev Neurosci 2012; 30:285-91.
11. Cannizzaro E, Martire M, Gagliano M, et al. Reversal of prenatal diazepam-induced deficit in a spatial-object learning task by brief, periodic maternal separation in adult rats. Behav Brain Res 2005;161:320-30.
12. Cannizzaro C, Plescia F, Martire M, et al. Single, intense prenatal stress decreases emotionality and enhances learning performance in the adolescent rat offspring: interaction with a brief, daily maternal separation. Behav Brain Res 2006;169:128-36.

Evaluation of antifouling activity of TiO_2 and Ag-doped TiO_2 in laboratory conditions to be applied on submerged archeological material

L. Mazza,[1] S.A. Ruffolo,[2] A. Macchia,[3] M.F. La Russa,[2] M. Barberio,[4] G.M. Crisci,[2] F. De Leo,[1] C.E. Urzì[1]

[1]Department of Biological and Environmental Sciences, University of Messina; [2]Department of Biology, Ecology and Earth Sciences, University of Calabria, Arcavacata di Rende (CS); [3]Syremont S.p.A., Arcavacata di Rende (CS); [4]Department of Physics, University of Calabria, Arcavacata di Rende (CS), Italy

The term of biofouling or biological fouling means the accumulation of microorganisms, plants, algae, or animals on wetted surfaces with consequent loss of the intrinsic characteristic of the material. Biofouling occurs worldwide in various submerged substrates of industrial interest as well as on materials that have an archeological and historical importance such as wood, metals and stones.[1] The process begins with the formation of a biofilm produced by bacteria or other microorganisms[2] and once it starts archeological materials are subjected to an increase of deterioration due to the grazing action of epilithic and endolithic marine micro- and macroorganisms. To avoid and/or to stop either micro or macro-biofouling processes, in marine environment conventional procedures include anti-fouling paints to prevent the attachment of sealife such as algae and molluscs.[3] However, these compounds slowly *leach* into the sea water, killing sealife, harming the environment and possibly entering in the food chain and in any case they cannot be directly applied on submerged archeological materials.[4] Hence, new products that are i) antifouling agents, ii) harmless to the valuable materials and iii) eco-friendly, are welcomed.[3,5] In the last ten years, Titanium oxides are proven to have antibacterial activity, if exposed to certain light wave lengths (photocatalytic activation). In particular, TiO2 is activated at UV wavelength, while Ag-TiO2 is photoactivated by visible spectrum and thus being effective also to a certain depth.[5] The aim of this study was to compare the antimicrobial activity of Titanium oxide (TiO2) and a Titanium oxide doped with Ag (Ag-TiO2) as described by Ruffolo and colleagues[5] against pure cultures of Gram positive and Gram negative bacteria as well as to evaluate the ability to interfere with the adhesion of bacteria on submerged treated marble slabs simulating a marine environment in laboratory conditions. For this purpose we carried out different sets of experiment in agarized medium and in UP distilled water to evaluate the antibacterial activity of the titanium oxides suspensions and the preventive effect against microbial adhesion on submerged material after 24-72 hrs. Laboratory experiments carried out on UP distilled water suspension showed that Ag-TiO2 activity was slightly higher than TiO2 (tested at concentration of 0.1 and 0.01%), perhaps due to the intrinsic antibacterial activity of the Ag alone. On marble slabs, both treatment with TiO2 and AgTiO2 were efficient to prevent the microbial colonization of the surfaces as shown by the SEM analysis. Untreated marble showed a diffuse presence of Extracellular Polymeric Substances (EPS) while on marbles slabs treated with TiO2 or TiO2-Ag or Ag no EPS production and no microbial colonization was evidenced. In conclusion, our preliminary results demonstrate that Titanium oxides could be successfully applied on the surface of submerged archeological items to prevent the initial microbial biofouling.

Correspondence: Clara Enza Urzì, Department of Biological and Environmental Sciences, University of Messina, viale F. Stagno d'Alcontres 31, 98166 Messina, Italy.
E-mail: urzicl@unime.it

Acknoweldgements: this research is supported by Italian national research project *COMAS* (Planned COnservation, *in situ*, of underwater archaeological artefacts).

References

1. Stanczak M. Biofouling: it's not just barnacles anymore. Available from: http://www.csa.com/discoveryguides/biofoul/overview.php
2. Gehrke T, Sand W. Interactions between microorganisms and physicochemical factors cause MIC of steel pilings in harbours. Houston, TX: NACE International; 2003.
3. Gomez de Saravia SG, Guiamet PS, Videla HA. Preventing biocorrosion without damaging the environment. Four innovative strategies. Northampton: Institute of Corrosion; 2001.
4. Ricci S, Pietrini AM, Bartolini M, Sacco Perasso C. Role of the microboring marine organisms in the deterioration of archaeological submerged lapideous artefacts (Baia, Naples, Italy). International Biodeterioration and Biodegradation (in press).
5. Ruffolo SA, Macchia A, La Russa MF, et al. Marine antifouling for underwater archaeological sites: TiO2 and Ag-doped TiO2. Int J Photoenergy 2013;2013:251647.

Poly ε-caprolactone scaffold in a rabbit critical size defects model: histological observations

V. Miragliotta,[1] G. Barsotti,[1] D. Puppi,[2] A. Coli,[1] M.R. Stornelli,[1] A. Briganti,[1] E. Giannessi,[1] C. Mota,[2] F. Carlucci,[1] F. Chiellini,[2] F. Dini[1]

[1]Department of Veterinary Sciences, University of Pisa; [2]Laboratory of Bioactive Polymeric Materials for Biomedical and Environmental Applications, Department of Chemistry and Industrial Chemistry, University of Pisa, Italy

Introduction

Repair of *critical size defects* (CSD)[1] remains a serious problem as the associated loss of function considerably impairs the quality of life of the affected patient.[2] Bone grafts remain an important part of orthopaedic surgeon's armamentarium to treat tissue loss related to disease or trauma.[3] Although autologous bone graft still represent the gold standard for CSD treatment, particularly for its properties of osteoinduction, osteogenesis, osteoconduction, histocompatibility and absence of immunogenicity, it presents undeniable drawbacks such as an elevated postoperative morbidity of donor site, thus requiring additional surgical procedure, with well-documented complication and discomfort for the patient.[4] Allogeneic bone graft, obtained from cadavers or living donors, is considered an alternative but for its limited regenerative capacity, risk of immunogenicity and rejection reactions, failure of vascularization, possibility of infection transmission or high cost of processing, its use is considered sub-optimal.[5] Bone graft substitutes, consisting of scaffolds of either synthetic or natural biomaterials, were developed: their ideal features are considered to be osteoconductivity, osteoinductivity, biocompatibility, biodegradability, and a structure similar to bone.[6] Here we report the effect of three-arm star branched poly(ε-caprolactone) (*PCL) developed as an anatomically-shaped scaffold (by the computer-aided wet-spinning technique[7,8] when implanted in a rabbit CSD model.

Materials and Methods

Eighteen healthy 4-month-old male New Zealand White rabbits, weighing between 2.0 and 3.0 kg, were included in the study under a protocol approved by the local ethic committee of Pisa University. Under general anaesthesia, a segmental defect of 20 mm (CSD) of the radial diaphysis with periosteum of the right forelimb was removed and substituted with *PCL scaffold. After surgery digital X-ray of treated forelimb was performed to evaluate length of osteotomy, and radiopacity of each scaffold. Bone regeneration was evaluated using a modified system to score defect bridging and bone formation on X-rays.[9,10] At 4, 8 and 12 weeks after surgery, specimens of 40 mm were collected (scaffold, 10 mm proximal and distal radius bone and ulna) and processed for histological evaluation. Haematoxilyn-Eosin, Mallory thricrome, Toluidine blue and Congo red stained sections of samples selected on the basis of their radiological score were examined.

Results and Discussion

The X-rays performed immediately after surgery confirmed that the defects were indeed CSD (20 mm±0.89). The epiphyseal plates at this time point were not completely closed. Mean assigned scores are reported in Table 1. Qualitative histological examination generally confirmed radiological observations. Sections obtained from samples with an high radiological score showed the presence of new-formed bone tissue invading the scaffold; some of them also showed the presence of a medullary canal occupied by fat cells and mononucleated elements resembling bone marrow cells (Figure 1a). The scaffold was never completely invaded or replaced by bone tissue that was always projecting from the radial margin of ulna (Figure 1b). In sections obtained from samples with a medium radiological score the new-formed bone tissue was present as a bridge projecting from ulna to the junctions between radius and the scaffold (Figure 1c). Ulnar periostium was always the main osteogenic source both in high and medium score samples (Figure 1d). Sections obtained from samples with a low radiological score did not show new-formed bone tissue (Figure 1e); connective/granulation tissue was present among the scaffold fibers together with some areas of mononucleated inflammatory infiltrate (Figure 1f).

Conclusions

*PCL guides tested in this study seem to be biocompatible: signs of rejection were never detected. The presence of a new-formed bone witness osteo-conductivity of the scaffold. Histological data showed that osteogenesis mainly arise from the periostium of ulna, initially reacting to form a bridge toward the scaffold. Further studies should investigate if modifications in the composition of the scaffold may increase its osteoconductivity and osteoinductivity.

Correspondence: Vincenzo Miragliotta, Department of Veterinary Sciences, University of Pisa, viale delle Piagge 2, 56124, Pisa, Italy.
E-mail: vincenzo.miragliotta@vet.unipi.it

Journal of Biological Research 2015; 88:5161

Table 1. Mean assigned scores and standard deviation values.

Time after surgery (weeks)	Mean assigned score	Standard deviation
4	7.75	3.8
8	9.33	2.71
12	7.66	4.72

References

1. Hollinger JO, Kleinschmidt JC. The critical size defect as an experimental model to test bone repair materials. J Craniofacial Surg 1990;1:60-8.
2. Schieker M, Mutschler W. Bridging post-traumatic bony defects. Established and new methods. Der Unfallchirurg 2006;109:715-32.
3. Soucacos PN, Johnson EO, Babis G. An update on recent advances in bone regeneration. Injury 2008;39(Suppl.2):1-4.
4. Dimitriou R, Jones E, McGonagle D, Giannoudis PV. Bone regeneration: current concepts and future directions. BMC Med 2011;9:66.
5. Finkemeier CG. Bone-grafting and bone-graft substitutes. J Bone Joint Surg Am 2002;84:454-64.
6. Puppi D, Chiellini F, Piras AM, Chiellini E. Polymeric materials for bone and cartilage repair. Prog Polymer Sci 2010;35:403-40.
7. Puppi D, Dinucci D, Bartoli C, et al. Development of 3D wet-spun polymeric scaffolds loaded with antimicrobial agents for bone engineering. J Bioact Compat Pol 2011;26:478-92.
8. Mota C, Puppi D, Dinucci D, et al. Additive manufacturing of star poly(ε-caprolactone) wet-spun scaffolds for bone tissue engineering applications. J Bioact Compat Pol 2013;28:320-40.
9. Hedberg EL, Kroese-Deutman HC, Shih CK, et al. Methods: a comparative analysis of radiography, microcomputed tomography, and histology for bone tissue engineering. Tissue Eng 2005;11:1356-67.
10. Bodde EW, Spauwen PH, Mikos AG, Jansen JA. Closing capacity of segmental radius defects in rabbits. J Biomed Mat A 2008;85:206-17.

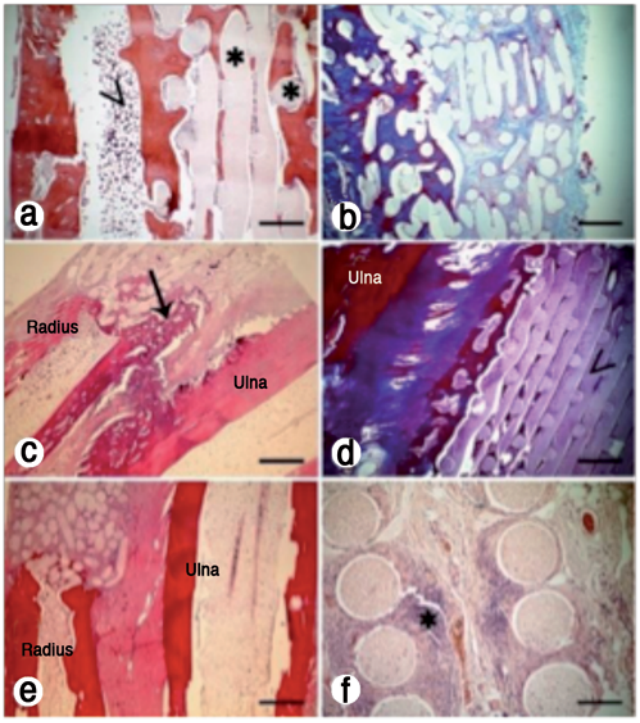

Figure 1. Representative photomicrographs of high-score (a, b) medium score (c, d) and low score (e, f) samples. a) Asterisks indicate the implanted scaffolds, while the arrowhead indicates the fat cells populating the new-formed medullary canal; scale bar=500 μm. b) The bone tissue (left) is growing into the implanted scaffold; scale bar 800 μm. c) The arrow indicates the new-formed bone as a bridge projecting from ulna to the junctions between radius and the scaffold; scale bar=2 μm. d) The ulnar periosteum is the main source of new-formed bone invading the scaffold (arrowhead); scale bar=800 μm. e) The scaffold (up-left) is not invaded by new-formed bone; scale bar=2 mm. f) The asterisk indicates the presence of mononucleated inflammatory cells; scale bar=200 μm.

Effect of thioridazine on erythrocytes

P. Modicano,[1,2] E. Lang,[2] M. Arnold,[2] R. Bissinger,[2] C. Faggio,[1] M. Abed,[2] F. Lang[2]

[1]Department of Biological and Environmental Sciences, University of Messina, Italy; [2]Department of Physiology, Eberhard-Karls-University of Tuebingen, Germany

Thioridazine, a phenothiazine drug with antipsychotic efficacy[1,2] and anti-microbial activity,[2] is particularly useful for the treatment of multidrug resistant tuberculosis.[3,4] This drug is known to trigger anemia.[5] At least in theory, the anemia could result from stimulation of suicidal erythrocyte death or eryptosis,[6] which is characterized by cell shrinkage, phospholipid scrambling of the cell membrane with phosphatidylserine exposure at the erythrocyte surface, increase of cytosolic Ca^{2+}-activity ($[Ca^{2+}]_i$) and activation of p38 kinase, a kinase expressed in human erythrocytes and activated by hyperosmotic shock, a known trigger of eryptosis.[7] The present study explored whether thioridazine triggers eryptosis. The concentrations required to trigger eryptosis are within the range of concentrations (6 μg/mL≈15 μM) encountered *in vivo*.[8] Since different erythrocyte specimens used in distinct experiments are differently susceptible to triggers of eryptosis, the same erythrocyte specimens have been used for control and experimental conditions. A 48 h exposure to thioridazine was followed by a significant: i) increase of $[Ca^{2+}]_i$ (30 μM), estimated from Fluo3-fluorescence in flow cytometry (Fluo-3/AM); ii) decrease cell volume, estimated from forward scatter (30 μM) in flow cytometry; iii) increase of the percentage of phosphatidylserine exposure, estimated from annexin-V-binding (≥12 μM) in flow cytometry (Annexin-V-FITC, 1:200 dilution); iv) increase of the percentage of hemolysed erytrocytes, estimated from the hemoglobin concentration in the supernatant This in turn was determined photometrically at 405 nm. The absorption of the supernatant of erythrocytes lysed in distilled water was defined as 100% hemolysis. As illustrated in Figure 1A, thioridazine treatment significantly increased the hemoglobin concentration in the supernatant. The percentage of hemolytic erythrocytes was, however, clearly smaller than the percentage of annexin V binding erythrocytes. Both, cell membrane scrambling and cell shrinkage could have resulted from increase of cytosolic Ca^{2+} activity ($[Ca^{2+}]_i$). However, even in the absence of extracellular Ca^{2+}, thioridazine still significantly increased the percentage of annexin-V-binding erythrocytes pointing to additional mechanisms involved. In order to explore whether the additional mechanisms could include p38 kinase, erythro-

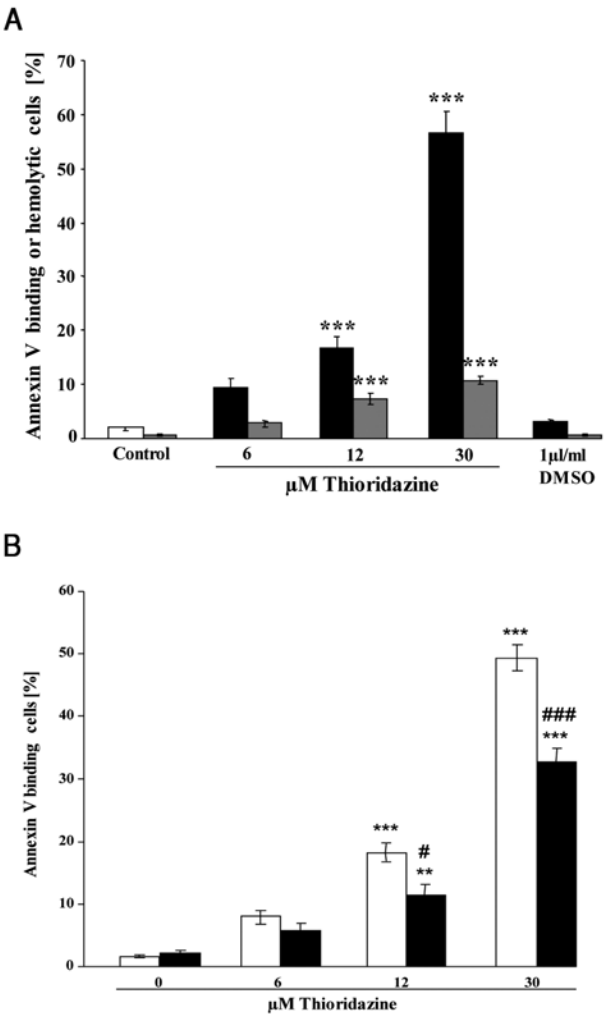

Figure 1. A) Effect of thioridazine on phosphatidylserine exposure and hemolysis. Arithmetic means±SEM (n=6) of erythrocytes annexin-V-binding following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) presence of thioridazine (6-30 μM). For comparison, arithmetic means±SEM (n=5) of the percentage of hemolysis is shown as grey bars. *(P<0.001) indicates significant differences from the absence of thioridazine (ANOVA). B) Effect of thioridazine on phosphatidylserine exposure in the presence or absence of p38 kinase inhibitor SB203580. Arithmetic means±SEM (n=6) of erythrocytes annexin-V-binding following incubation for 48 h to Ringer solution without or with presence of thioridazine (6-30 μM) in the absence (white bars) or presence (black bars) of 2 μM SB203580. **(P<0.01); ***(P<0.001) indicates significant differences from the absence of thioridazine (ANOVA); #(P<0.05); ###(P<0.001) indicates significant differences from the absence of SB203580 (ANOVA).**

Correspondence: Paola Modicano, Department of Physiology, Eberhard-Karls-University of Tuebingen, Gmelinstrasse 5, 72076 Tuebingen, Germany.
E-mail: paolamodicano@libero.it

Journal of Biological Research 2015; 88:5161

cytes were exposed in further experiments to 6-30 μM thioridazine for 48 h in either the presence or absence of the p38 kinase inhibitor SB203580 (2 μM) (Figure 1B). In conclusion, thioridazine stimulates eryptosis and is partially effective by activation of p38 kinase and by increase of cytocolic Ca^{2+} activity. Nominal absence of extracellular Ca^{2+} and p38 kinase inhibitor SB203580 significantly blunted but did not abolish annexin-V-binding following thioridazine exposure.

References

1. Purhonen M, Koponen H, Tiihonen J, Tanskanen A. Outcome of patients after market withdrawal of thioridazine: a retrospective analysis in a nationwide cohort. Pharmacoepidem Dr S 2012; 21:1227-31.
2. Thanacoody RH. Thioridazine: the good and the bad. Recent Pat Anti-Inf 2011;6:92-8.
3. Amaral L. Thioridazine: an old neuroleptic effective against totally drug resistant tuberculosis. Acta Mèdica Portuguesa 2012;25:118-21.
4. Amaral L, Viveiros M. Why thioridazine in combination with antibiotics cures extensively drug-resistant Mycobacterium tuberculosis infections. Int J Antimicrob Ag 2012;39:376-80.
5. King DJ, Wager E. Haematological safety of antipsychotic drugs. J Psychopharmacol 1998;12:283-8.
6. Lang F, Gulbins E, Lerche H, et al. Eryptosis, a window to systemic disease. Cell Physiol Biochem 2008;22:373-80.
7. Gatidis S, Zelenak C, Fajol A, et al. p38 MAPK activation and function following osmotic shock of erythrocytes. Cell Physiol Biochem 2011;28:1279-86.
8. Gottschalk LA, Biener R, Noble EP, et al. Thioridazine plasma levels and clinical response. Compr Psyc 1975;16:323-37.

Microscopia elettronica a scansione per l'analisi morfologica e ultrastrutturale di cariossidi di frumento duro

I. Garaguso,[1] M. Giammanco,[2] R. Acquistucci,[1] D. Di Majo,[2] F. Nobili[1]

[1]Council Research and Experimental Center in Agriculture-Research Center for Food and Nutrition, Rome; [2]Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, Italy

Introduzione

Questo studio è stato svolto nell'ambito del Progetto *ALISAL*: *Miglioramento delle proprietà igienico-sanitarie, salutistiche e funzionali di materie prime per l'alimentazione dell'uomo e/o degli animali*, promosso dal Ministero delle Politiche Agricole, Alimentari e Forestali italiano (MIPAAF). Lo studio in oggetto è parte del progetto sopra indicato il cui scopo era quello di selezionare nuovi genotipi di cereali, in particolare di frumento duro, al fine di migliorare le caratteristiche nutrizionali e tecnologiche dell' intera filiera produttiva. La fase preliminare dello sviluppo progettuale è stata quella di sviluppare un metodo per la caratterizzazione morfologica e ultrastrutturale delle granelle d'interesse allo scopo di fornire indicazioni qualitative e quantitative per la precisa caratterizzazione dei campioni. I risultati ottenuti sono di seguito riportati.

Materiali e Metodi

Al fine di evidenziare eventuali differenze morfologiche e ultrastrutturali tra i campioni, le cariossidi di frumento duro sono state analizzate tal quali mediante microscopia elettronica a scansione in ambientale e a pressione variabile utilizzando il microscopio SEM EVO LS10 ZEISS. Questo microscopio è dotato di una sorgente di elettroni LaB6 in grado di fornire una luminosità del fascio elettronico uniforme, stabile e di conseguenza un alto contrasto; tali caratteristiche lo rendono particolarmente adattato all'osservazione di campioni sia disidratati che umidi. Sulle cariossidi di grano duro si è eseguito un adeguato taglio sagittale e le sezioni *tal quali,* senza alcuna preparativa sono state fissate su gli appositi *stub*, successivamente si è passati all'osservazione predisponendo il microscopio a pressione variabile ed utilizzando il detector CZBSD come rivelatore primario e il detector SE1 come rivelatore secondario. Il mix dei due ci ha fornito una precisa mappa ultrastrutturale di ogni cariosside testata. Secondo le specifiche progettuali abbiamo preso in considerazione tre cultivar di frumento duro coltivate nel Sud Italia (Sicilia) negli anni 2010 e 2011. I campioni sono stati classificati come SA_1(*cultivar Sant'Agata*), DU_1(*cultivar Duilio*), SI_1(cultivar *Simeto*) per le produzioni dell'anno 2010 e SA_2, DU_2, SI_2 per le produzioni dell'anno 2011.

Risultati e Discussione

I campioni di grano duro classificati, come detto in precedenza, sono stati sottoposti all'analisi in scansione elettronica che è stata eseguita in due fasi. Una prima fase è stata finalizzata allo studio della superficie delle cariossidi a basso ingrandimento per rilevare le caratteristiche morfologiche più grezze quali la lunghezza e la larghezza media (Figura 1).

La seconda serie di osservazioni è stata realizzata ad alto ingrandimento in modo da evidenziare le componenti ultrastrutturali delle cariossidi, quali lo strato aleuronico, la camera embrionale, e la morfologia dell'endosperma, cercando per ogni campione di fare le osservazioni in posizioni di coordinate analoghe e quindi confrontabili. Non è stata rilevata alcuna variazione morfo-ultrastrutturale significativa per la maggior parte dei campioni: tuttavia nei campioni DU_2 (Figura 2B) *cultivar Duilio* produzione del 2011 e nel campione SA_1 (Figura 2A) *cultivar Sant'Agata* produzione del 2010, abbiamo osservato una serie di modificazioni di notevole entità. Nello specifico il campione DU_2 presenta una forte discrasia fra cariossidi dello stesso campione e, comunque, le

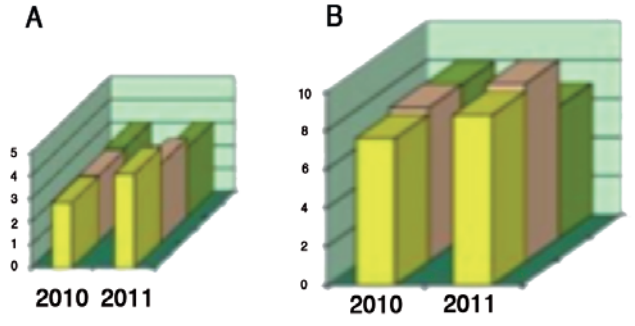

Figura 1. Misura della larghezza (A) e della lunghezza (B) (in mm) delle cariossidi dei frumenti Sant'Agata (giallo), Simeto (arancio), Duilio (verde).

Correspondence: Fabio Nobili, Council Research and Experimental Center in Agriculture-Research Center for Food and Nutrition, via Nazionale 82, 00184 Rome, Italy.
E-mail fabio.nobili@entecra.it

Journal of Biological Research 2015; 88:5161

dimensioni dei parametri macroscopici, come si evidenzia nella Figura 1, sono inferiori a tutti gli altri campioni testati. Le dimensioni volumetriche ridotte e coartate dell'endosperma (Figura 2B) fanno presupporre che il suddetto campione abbia subito uno stress da temperatura in fine stagione o una conservazione anomala. Il campione SA_1 (Figura 2A) invece presenta le cariossidi più omogenee fra loro, mentre presenta lo strato aleuronico iperplasico (Figura 2A) in contrasto con quello della normale morfologia descritto in letteratura. Un'ipotesi è che tale campione abbia subito uno stress da temperatura in un momento precoce di crescita e che abbia poi innescato successivamente un parziale recupero realizzando una iperplasia delle cellule aleuroniche in maniera di aumentare la produzione di amido primaria e compensare il *gap* di crescita iniziale.

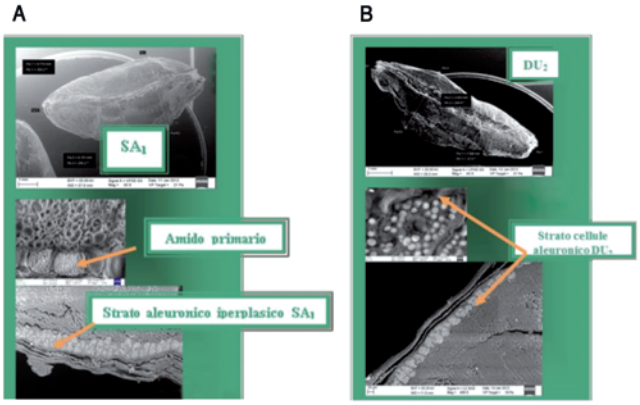

Figura 2. Campioni SA_1 (A) *cultivar Sant'Agata* produzione del 2010 e DU_2 (B) *cultivar Duilio* produzione del 2011.

Conclusioni

Questo studio preliminare ci ha permesso di caratterizzare morfologicamente i campioni di grano duro fornitoci e ci ha consentito di individuare il campione più performante dal punto di vista morfologico e ultrastrutturale che è risultato essere il grano duro della *cultivar Sant'Agata*. La tecnica utilizzata in questo lavoro (microscopia elettronica a scansione) può rappresentare un valido supporto per caratterizzare campioni biologici con struttura definita e parametrizzabile, in questo caso grano duro, da destinare a ulteriori indagini di natura analitico- funzionale.

Biological evaluation of the action of *Undaria pinnatifida* extract on equine red bloods cells

M. Pagano,[1] G. Genovese,[1] M. Morabito,[1] S. Casella,[2] C. Faggio[1]

[1]Department of Biological and Environmental Sciences, University of Messina; [2]Department of Veterinary Sciences, University of Messina, Italy

Recently, a great deal of interest has been developed to isolate novel bioactive compounds from marine resources because of their numerous health beneficial effects. Among marine resources, algae are valuable sources of structurally diverse bioactive compounds. The cell walls of algae are rich in polysaccharides, some of which sulphated (SPs) such as fucoidans in brown algae, carrageenans and agar in red algae and ulvans in green algae. These SPs exhibit many beneficial biological properties such as anticoagulant, antiviral, antioxidative, anticancer and immunomodulating activities. Therefore, they have great potential for further development as products in nutraceutical, pharmaceutical and cosmetics areas.[1]

Biological activities of SPs depend on chemical structure, molecular weight and chain conformations. The fucoidans are polysaccharides containing relevant percentages of L-fucose and sulfate ester groups[2] (Figure 1).

Fucoidans display several physiochemical and biological features of potential interest for food, pharmaceutical, agricultural and chemical applications.[3]

Among the properties of algal polysaccharides, the anticoagulant activity of collecting so much scientific interest in this direction are being conducted extensive research. Fucans have activities similar to those of heparin, a drug for excellence with anticoagulant action.[4]

In the veterinary field, one of the diseases that can affect the species *Equus caballus* the most severe and debilitating, it is certainly laminitis, an inflammation of the laminae of the foot. The affection of the foot is only a local manifestation of a systemic metabolic disorder that affects the cardiovascular apparatus, endocrine system and urinary tract, blood clotting and the acid-base balance. It is believed that the basis of the decrease in capillary blood flow and ischemic necrosis jobs are dynamic vessels mangled and bleeding disorders.[5] To this between the various drugs is used heparin, for its anticoagulant action.

In recent years fucoidans have been investigated to develop novel drugs.

In this study was evaluated the toxicity of algal extracts from brown algae *Undaria pinnatifida*. The polysaccharides extracts were tested on equine red blood cells to evaluate potential haemolytic effects on cell membranes. *Undaria pinnatifida* samples were collected in the lagoon of Venice in May of 2011, the component epiphytic clean, dried in the sun for several days and finally crushed. The sample was treated with 100% ethanol and placed in an oven at 70°C for one hour, it was centrifuged at 4000 rpm for 10 minutes and treated with 100% acetone to obtain a complete depigmentation. Then the material was centrifuged at 4000 rpm for 10 minutes at room temperature.

The pellet (dispersed in distilled water) was placed in an oven at 70°C for 24 hours to allow the passage of the polysaccharides in solution. The solution was centrifuged at 4000 rpm for one hour at room temperature. The supernatant was mixed with ethanol 96% with a ratio V/V (volume of sample equal to the volume of ethanol). The precipitate, consisting of the crude polysaccharides, has been exposed to complete dehydration at room temperature and then pulverized by the use of a pestle.

Equine blood samples were collected from five healthy donators, were drawn into syringes filled with sodium citrate as an anticoagulant. The algal extracts was dissolved in a buffer, a saline solution at pH 7.4 with the following composition (mM): 125 NaCl, 5 KCl, 1 $MgSO_4$, 32 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5 D- (+)-glucose, 1 $CaCl_2$; pH 7.4. Algal extracts was tested at two different concentrations 10μg/ml and 20μg/ml. In all studies, using horse red blood cells, control experiments were carried out without extract. We were evaluated the toxicity of algal extracts through two tests: Trypan blue test and hemolysis test. In order to evaluate the toxicity of each sample i) by Trypan blue test, the cells were counted in Burker's chamber and the

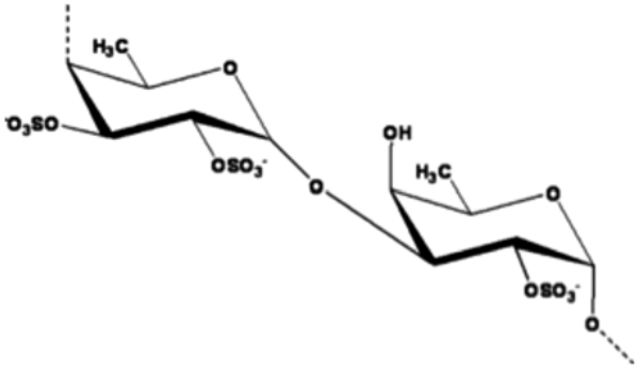

Figure 1. Chemical structure of the repeating dimeric units of fucoidan.

Correspondence: Maria Pagano, Department of Biological and Environmental Sciences, University of Messina, viale Fernando Stagno d'Alcontres 31, 98166 Messina, Italy.
E-mail: mariapagano88@virgilio.it

Journal of Biological Research 2015; 88:5161

Table 1. Percentage of hemolysis in control and after the additional of *Undaria pinnatifida* polisaccaride extract.

	300mOsm	200mOsm	150mOsm	100mOsm	0mOsm
Control	0	0	9	85	100
10 μg/1mL	0	0	10	84	100
20 μg/1mL	0	0	9	81	100

cells were expressed as percentages of viable cells; ii) the hemolysis test to see the hemolytic effects of the algal extracts, that evaluates hemoglobin release in the plasma following molecule. After incubation at room temperature, the tubes were centrifuged (5 minutes at 1500 rpm) and the supernatants were determined photometrically with a spectrophotometer at the absorption of hemoglobin (540 nm). As a measure of hemolysis, Hb concentration of the supernatants was determined photometrically. The absorption of the supernatant of erythrocytes lysed in distilled water was defined as 100% haemolysis. In the treatment of RBCs with polysaccharides extracted from *Undaria pinnatifida,* hemolysis occurence at identical concentrations of the control conditions suggest that the compounds does not shows toxicity effects in equine red blood cells at the concentration tested (Table 1).

Currently in the lagoon of Venice *Undaria pinnatifida* is removed and treates as waste stored in landfills and incinerated. This study shows a possible exploitation of *Undaria pinnatifida*, not toxic to equine red blood cells, as a source of anticoagulant drug with the aim of trasforming a waste into a valuable biomass.

References

1. Wijesekara I, Pangestuti R, Kim S-K. Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. Carbohydrate Polymers 2011;84:14-21.
2. Li B, Lu F, Wei X, Zhao R. Fucoidan: structure and bioactivity. Molecules 2008;13:1671-95.
3. Lahaye M, Robic A. Structure and functional properties of ulvan, a polysaccharide from green seaweeds. Biomacromolecules 2007;8: 1765-74.
4. Costa L, Fidelis GP, Cordeiro SL, et al. Biological activities of sulfated polysaccharides from tropical seaweeds. Biomed Pharmacother 2010;64:21-8.
5. Van Eps AW. Studies on the pathogenesis and prevention of equine laminitis. PhD Diss. St. Lucia: School of Veterinary Science, The University of Queensland ed.; 2007.

Nutritional quality of extra virgin olive oil determined by the application of a new functional matematic index

D. Di Majo,[1] E. Finotti,[2] M. La Guardia,[3] L. La Neve,[1] S. Palumbo,[1] M. Giammanco[1]

[1]Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo; [2]Council Research and Experimental Center in Agriculture-Research Center for Food and Nutrition, Rome; [3]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo

Introduction

The extra virgin olive oil is very important in the Mediterranean diet. From the nutritional point of view, the high energy content and an optimal ratio between saturated and unsaturated fatty acids, together with the presence of minor compounds such as phenols and tocopherols and the related antioxidant capacity, confer to this product a high healthy value.[1] The extra-virgin oil's nutritional properties depend on the pedoclimatic conditions, cultivar and the production process. In the last years many studies have been performed to remark and describe the safety and nutritional quality of the extra-virgin olive oils but it is difficult to compare different parameters representative of oil's nutritional quality and to establish which of them is more important to define the nutritional value of an oil. In 2007, Finotti and colleagues[2] developed a Functional Mathematic Index (FMI) in order to quantify the nutritional quality of extra virgin olive oil and then the same authors applied it at other foods, such as tea,[3] potatoes.[4] This index is important because it is able to convert a concept as the *quality* into a numeric quantity. The FMI takes into account different nutritional, antioxidant and chemical parameters representative of oil properties. These parameters, suitably processed with the *FMI Workbench* free software, provide a unique value indicated as *global* quality index (I_{GQ}). We have calculated both the global FMI and the local FMIs. Aim of this study is to compare the nutritional properties of extra virgin olive oils from different olive varieties, Biancolilla and Oglialora grown in Sicily, by the functional mathematic index.[5]

Correspondence: Danila Di Majo, Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo, viale delle Scienze, Ed. 16, 90127 Palermo, Italy.
E-mail: danila.dimajo@unipa.it

Journal of Biological Research 2015; 88:5161

Materials and Methods

Samples of extra-virgin olive oils from two different cultivar (Biancolilla, Oglialora) and processed by different extraction methods (continuous cycle and hydraulic press) have been analyzed. The drupes are grown in the same climatic conditions. The parameters analyzed to calculate the FMI have been divided in three groups which are related to chemical, nutritional, technologic properties of oil. The three groups are: i) nutritional parameters: fatty acids (numbers from 1 to 4; Table 1); ii) chemical parameters: acidity and peroxide number (numbers 5 and 6; Table 1); iii) technological process parameters: lipidic antioxidant capacity, tocopherols and total phenols (numbers 7-9; Table 1). The results of the analyses were processed by the software and the I_{GQ} and LQ of each oil sample have been compared in order to define which of them had the better nutritional properties. The FMI varies from zero to one (good to poor). The global index has been expressed as *goodness percentage* (%FMI). The upper and the lower bounds for each group are established and they are reported in Table 1.

Results and Discussion

The analyses allow to classify all oil samples as extra-virgin olive oils. All parameters are within the limits of the Italian (E.U.) law and those established in Table 1. No difference among two cultivars about acidity, peroxide number and fatty acids profile was found. The lipofilic antioxidant capacity was higher in Biancolilla variety than in Oglialora with both extractive methods (P=0.0001); sample oils extracted with continuous method have shown a lower of 16% content of tocopherols for both variety.

The FMI of all oil samples varies from 0.27 to 0.34, therefore they satisfy the necessary conditions to be considered high quality extra-virgin oil; Nonetheless, the cultivar Biancolilla has shown a global index better than the Oglialora. From the study of local FMI we observed that the nutritional quality of oils extracted by continuous cycle is penalized in the total polyphenols and lipofilic antioxidant capacity parameters.

Conclusions

The FMI allows to detect that the Biancolilla variety is better than Oglialora and then to underline which parameter can be modified in order to increase the nutritional quality of the oil analyzed.

Table 1. Parameters and methods used to analyse the extra-virgin oil samples. Upper and lower bounds for each parameters established following Finotti and colleagues.[2]

Index number	Parameters	Method used to analyze and related references	Lower bound	Upper bound
1	Palmitic acid (C16:0)	Chromatographic analyses (EU Regulation 2568/91, All. Xa and All. Xb)	7,5%	20%
2	Stearic acid (C18:0)		0,5%	5%
3	Oleic acid (C18:1)		55%	83%
4	Linoleic acid (C18:2)		3,5%	21%
5	Acidity	EU Regulation 2568/91, all. II	0%	0,8%
6	Peroxide number	EU Regulation 2568/91, all. III	0 meq/O_2	20 mEq/O_2
7	Lipidic antioxidant capacity	(Finotti and colleagues 1998,2000)	-1,6 K_a/K_c	+ 1,6 K_a/K_c
8	Tocopherol	HPLC, detector UV/Vis (method of SSOG)	1,2 mg/100g	43mg/100g
9	Total phenols	HPLC, detector UV/Vis (method of SSOG)	20 mg/kg	900 mg/kg

References

1. Tripoli E, Giammanco M, Tabacchi G, et al. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. Nutr Res Rev 2005;18:98-112.
2. Finotti E, Bersani AM, Bersani E. Total quality indexes for extra-virgin olive oils. J Food Quality 2007;30:911-33.
3. Finotti E, Bersani E, Friedman M. Application of a Functional Mathematical Index for Antibacterial and Anticarcinogenic Effects of Tea Catechins. J Agric Food Chem 2011;59:864-9.
4. Finotti E, Bersani E, Del Prete E, Friedman M. A functional mathematical index for predicting effects of food processing on eight sweet potato (Ipomoea batatas) cultivars. J Food Comp Anal 2012;27:81-6.
5. Finotti E, Bersani E, Bersani AM, et al. Variation of nutritional parameters in the olive oil during the ripening phase. La Rivista di Scienze dell'Alimentazione 2011;2:33-41.

Attività antibatterica di estratti algali nei confronti di batteri isolati da tamponi auricolari

G. Pane,[1] E. Coppo,[2] G. Cacciola,[2] E. Giacco,[3] G.L. Mariottini,[3] A. Marchese[2]

[1]Nose and Throat Unit, Department of Surgical and Integrated-Diagnostic Sciences, University of Genoa; [2]Microbiology Unit, Department of Surgical and Integrated-Diagnostic Sciences, University of Genoa; [3]Department of Earth, Environmental and Life Sciences, University of Genoa, Italy

L'otite esterna consiste in un processo infiammatorio del condotto uditivo che può giungere ad interessare anche la membrana timpanica. Questa patologia, i cui sintomi più caratteristici sono otorrea ed otalgia, è causata generalmente da infezioni batteriche e, più raramente, da infezioni micotiche;[1] i principali microrganismi responsabili sono *Pseudomonas aeruginosa* e *Staphylococcus aureus*.[2] La diagnosi di otite esterna si basa, oltre che sull'esame clinico, sull'effettuazione di tamponi auricolari che, permettendo il riconoscimento dell'agente eziologico, costituiscono il supporto indispensabile per una corretta terapia; quest'ultima si basa essenzialmente sulla somministrazione di antibiotici, eventualmente associati ad antinfiammatori steroidei. Tuttavia, le infezioni da *P.aeruginosa* e da *S.aureus* pongono seri problemi terapeutici legati alla farmaco-resistenza, particolarmente sviluppata in ambiente ospedaliero, che in molti ceppi si estende alla maggior parte degli antibiotici in uso. Nell'ambito degli studi orientati alla scoperta e all'utilizzazione di sostanze ad attività antibiotica, interessanti molecole sono state isolate in microalghe;[3,4] inoltre, si è osservato che la somministrazione di cellule algali favorisce l'attivazione dei macrofagi e l'aumento della produzione di cellule staminali nel midollo osseo accelerandone la differenziazione in cellule immunocompetenti.[4,5] Questo studio intende valutare la popolazione microbica del condotto uditivo in pazienti affetti da otite esterna e verificare la sensibilità dei microrganismi responsabili ad estratti algali opportunamente approntati. Per l'indagine microbiologica sono stati utilizzati tamponi auricolari prelevati da 100 pazienti con presunta otite acuta esterna. Ogni tampone è stato seminato in opportuni terreni di coltura selettivi per Gram- (Columbia horse blood agar, Mc Conkey Agar), per stafilococchi (Mannitol Salt Agar) e per miceti (Sabouraud Agar); le piastre sono state tutte incubate a 37°C per 18/24 ore ad eccezione di quelle con Sabouraud per le quali l'incubazione è stata prolungata fino ad 1 settimana. I microrganismi sono stati identificati mediante esame microscopico con colorazione di Gram. In seguito, per i ceppi Gram+ sono stati eseguiti il test della catalasi, per discernere tra *Staphylococcaceae* (catalasi positive) e *Streptococcaceae* (catalasi negative) ed il test della coagulasi, per discernere tra *S. aureus* (coagulasi positivo) e gli altri stafilococchi (coagulasi negativi). L'attività antibiotica di estratti algali è stata verificata utilizzando colture della specie marina *Dunaliella tertiolecta* Butcher 1959 (Chlorophyceae) e della specie d'acqua dolce *Pseudokirchneriella subcapitata* (Chlorophyceae). L'estratto algale è stato preparato per centrifugazione; il pellet è stato lavato con tampone fosfato (per *P. subcapitata*) o con PBS (per *D. tertiolecta*), è stato successivamente sottoposto a 20 cicli di sonicazione di 30 secondi ciascuno e a successiva centrifugazione (3500 rpm per 30 minuti). Al pellet ottenuto è stato aggiunto metanolo al 60% (0,25 g/mL) e si è proceduto ad una nuova centrifugazione (3500 rpm per 20 minuti); il sopranatante ottenuto è stato filtrato (0,22 µm - Millipore GV) ottenendo l'estratto che è stato utilizzato nello studio. Colture batteriche pure in fase di crescita esponenziale (5×10^5 CFU/mlL) ottenute da tamponi auricolari raccolti tra gennaio 2012 e gennaio 2013 presso il Pronto Soccorso dell'IRCCS Azienda Ospedaliera Universitaria San Martino IST da 100 pazienti (di cui 58,06% maschi e 41,94% femmine) di età compresa tra 15 e 92 anni (media=48,15 anni) sono state trattate con diverse concentrazioni di estratti algali di *P. subcapitata e D. tertiolecta*. L'attività antimicrobica degli estratti è stata determinata dopo 18-24 ore di incubazione ed è stata calcolata la Concentrazione Minima Inibente (MIC) secondo metodiche standardizzate;[6] 84 tamponi sono risultati positivi per la presenza di uno o più microrganismi (Tabella 1). *Pseudomonas aeruginosa* e *Staphylococcus aureus* sono risultati i patogeni più ricorrenti;

Tabella 1. Microrganismi isolati dai tamponi positivi.

	Ceppo	n. ceppi isolati	%
Gram+	*Staphylococcus aureus*	14	11,20
	Stafilococchi coagulasi-negativi (*)	22	17,60
	Enterococcus spp.	1	0,80
	Kokuria spp.	2	1,60
	Streptococcus pneumoniae	2	1,60
	Micrococcus spp.	8	6,40
Gram-	*Pseudomonas aeruginosa*	31	24,80
	Escherichia coli	8	6,40
	Klebsiella spp. (^)	7	5,60
	Altri enterobatteri (§)	7	5,60
	Altri Gram- non fermentanti (#)	10	8,00
	Vibrionaceae	2	1,60
	Miceti (°)	11	8,80

**S. epidermidis* (14), *S. capitis* (4), *S. xylosus* (2), *S. haemolyticus* (1), *S. symulans* (1); ^*K. pneumoniae* (5), *K. oxytoca* (2); §*P. mirabilis* (2), *E. cloaceae* (2), *E. aerogenes* (1), *R. terrigena* (1), *S. marcescens* (1); #*W. paucula* (2), *A. xylosoxydans* (2), *A. baumannii* (1), *P. alcaligenes* (1), *B. cepacia* (1), *R. picketti* (1), *S. paucimobilis* (1), *A. hydrophylia* (1); °*Candida* spp. (6), *Aspergillus niger* (5).

Correspondence: Erika Coppo, Microbiology Unit, Department of Surgical and Integrated-Diagnostic Sciences, University of Genoa, largo Benzi 10, 16132 Genoa, Italy.
E-mail: erika.coppo@unige.it

Journal of Biological Research 2015; 88:5161

le micosi hanno avuto un'incidenza molto inferiore (<9 %). I valori di MIC ottenuti trattando ceppi di *P.aeruginosa* e di *S. aureus* con estratto da cellule di *D. tertiolecta* sono risultati compresi rispettivamente tra $1,4\times10^9$ e $5,6\times10^9$ cell./mL e tra $2,8\times10^9$ e $1,1\times10^{10}$ cell./mL. I valori di MIC dei ceppi sottoposti all'estratto da cellule di *P. subcapitata* sono risultati compresi tra $6,2\times10^9$ e $1,2\times10^{10}$ cell./mL per P. aeruginosa e tra $1,6\times10^9$ e $1,2\times10^{10}$ cell./mL per *S. aureus*.

Questo studio ha confermato che *P. aeruginosa* e *S. aureus* sono gli agenti principali responsabili delle otiti esterne. Nel complesso, *P. aeruginosa* è prevalente e presenta una maggiore incidenza nei mesi estivi. Gli estratti provenienti da *D. tertiolecta* e *P. subcapitata* hanno mostrato attività antibatterica, in particolare nei confronti di *P. aeruginosa*, (MIC90=5,6×109 e 6,2×109 cell./mL rispettivamente) ed un minore effetto nei confronti di *S. aureus* (MIC90=1,12×1010 e 1,25×1010 cell./mL rispettivamente). Considerate le difficoltà derivanti dalla presenza di ceppi resistenti, l'individuazione di nuove molecole bioattive di origine naturale può costituire una prospettiva terapeutica di notevole interesse. In questo ambito, i risultati ottenuti indicano che estratti da specie algali fitoplanctoniche possono risultare adatti per ottenere sostanze ad attività antibiotica utili per il trattamento di patologie otorinolaringoiatriche causate da agenti microbici.

Bibliografia

1. Sander R. Otitis externa: a practical guide to treatment and prevention. Am Fam Physician 2001;63:927-36.
2. Schaefer P, Baugh RF. Acute otitis externa: an update. Am Fam Physician 2012;86:1055-61.
3. Reichelt JL, Borowiyzka MA. Antimicrobial activity from marine algae: results of a large scale screening program. Hydrobiology 1984;116/117:158-67.
4. Al-Haj NA, Mashan NI, Shamsudin MN, et al. Antibacterial activity in marine algae Eucheuma denticulatum against Staphylococcus aureus and Streptococcus pyogenes. Research Journal of Biological Sciences 2009;4:519-24.
5. Guzman S, Gato A, Lamela M, et al. Anti-infiammatory and immunomodulatory activities of polysaccharide from Chlorella stigmatophora and Phaeodactylum tricornutum. Phytother Res 2003;17:665-70.
6. CLSI. Performance Standards for antimicrobial susceptibility testing; Sixteenth Informational Supplement. Approved Standard M100-S18. Clinical and Laboratory Standards Institute, Wayne, PA, USA; 2008.

Relazione fra l'ipertensione e le cadute accidentali: i potenziali effetti positivi dell'esercizio fisico sulla pressione sanguigna

A. Patti,[1] C. Mammina,[2] M.C. Cataldo,[3] M.A. Montalto,[1] T. Alden,[4] R. Palma,[1] A. Bianco,[1] A. Palma[1]

[1]Sport and Exercise Science Research Unit, University of Palermo; [2]Department of Health Promotion G. D'Alessandro, University of Palermo; [3]National Health Service, ASP 6, Palermo, Italy; [4]School of Science, University of Greenwich at Medway, UK

Introduzione

La letteratura scientifica supporta la relazione fra le variazioni di pressione sanguigna e il fenomeno cadute nell'anziano; in particolare l'ipotensione è associata a perdita di equilibrio. Una diminuzione di 20mmHg o più della pressione sistolica o una diminuzione di 10mmHg o più in diastolica sono associati a perdita di equilibrio e cadute. Altri studi suggeriscono una relazione fra l'ipertensione e l'ipotensione e in definitiva supportano l'idea che il rischio di cadute sarebbe più alto nelle persone con ipertensione.[1] Lo scopo del nostro studio è capire e valutare, tramite una scala validata, se uno stile di vita attivo influisce significativamente nell'equilibrio residuo in soggetti ipertesi.

Materiali e Metodi

La Berg Balance Scale (BBS) è stata somministrata a due gruppi di soggetti anziani che vivono nella zona città di Palermo, Italia. Il primo gruppo era costituito da centododici soggetti che riferivano di essere affetti da ipertensione o esserne in cura (HP-G); il secondo gruppo consisteva in novantasei soggetti che dichiaravano di non soffrire di tale patologia (NP-G). I criteri di esclusione sono stati: i) persone con un'età inferiore a 65 anni; ii) persone con una diagnosi positiva di malattie fortemente debilitanti; iii) atleti ex professionisti. I punteggi sono stati utilizzati per correlazioni statistiche.

Correspondence: Antonio Palma, Sport and Exercise Science Research Unit, University of Palermo, viale delle Scienze 15, 90128, Italy.
E-mail: antonio.palma@unipa.it

Journal of Biological Research 2015; 88:5161

Risultati

Duecentotto persone hanno partecipato allo studio. 112 nel gruppo HP-G (Età: 74±8,12 anni; Peso 70,05±11,51; Altezza 162,58±8,12), 96 nel gruppo NP-G(Età: 72,5±7,62 anni; Peso 67,89±12,06; Altezza 159,75±9,60). I valori di BBS (Figura 1) erano nel NP-G 47,14±11,53; mentre nel HP-G erano 39,96±14,90 (P<0,0001). Inoltre, il 52% di HP-G ha riferito di praticare una regolare attività fisica. I soggetti attivi di HP-G (Figura 2) hanno mostrato valori pari a 48,12±9,08, mentre i soggetti sedentari di HP-G hanno registrato valori pari a 31,19±14,99 (P<0,0001).

Discussione

L'attività motoria sembra influire positivamente sull'equilibrio residuo dei soggetti analizzati. I risultati suggeriscono che soggetti iperte-

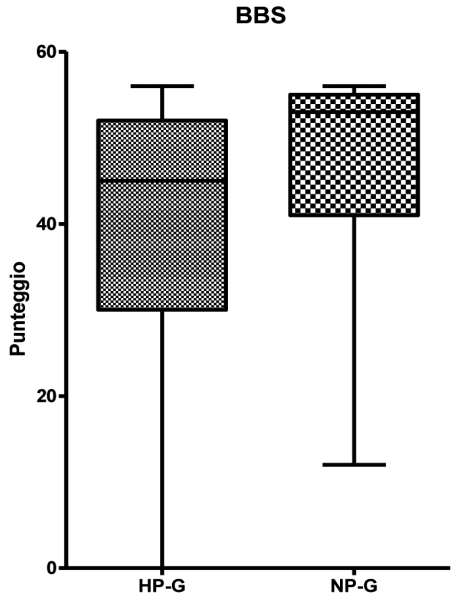

Figura 1. Valori della Berg Balance Scale in soggetti affetti da ipertensione o in cura (HP-G) e in soggetti non affetti da ipertensione (NP-G).

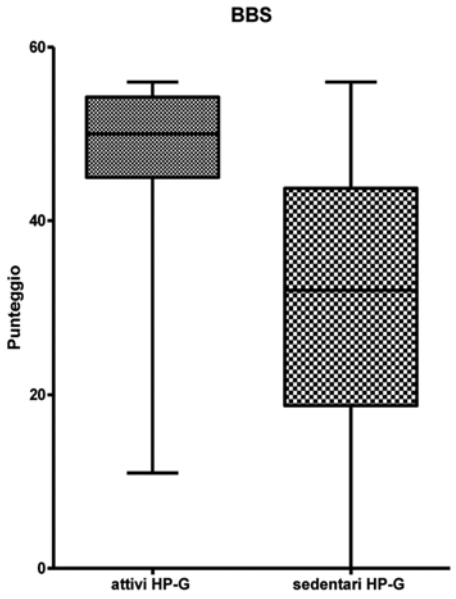

Figura 2. Valori della Berg Balance Scale in soggetti affetti da ipertensione o in cura (HP-G) attivi e sedentari.

si dovrebbero partecipare a programmi di attività motoria volti a prevenire le cadute. Sarebbe interessante stimare con ulteriori studi se l'attività motoria agisca direttamente sui meccanismi fisiologici emodinamici[2] o migliora semplicemente la performance atletica agendo così sull'equilibrio residuo.

Bibliografia

1. Gangavati A. Hypertension, orthostatic hypotension, and the risk of falls in a community-dwelling elderly population: the maintenance of balance, independent living, intellect, and zest in the elderly of Boston study. J Am Geriatr Soc 2011;59:383-9.
2. Pal S, Radavelli-Bagatini S, Ho S. Potential benefits of exercise on blood pressure and vascular function. J Am Soc Hypertens 2013;7:494-506.

Espressione genica delle citochine nella tunica albuginea in soggetti affetti da malattia di La Peyronie. Studio pilota con gruppo di controllo

C. Pavone,[1] G. Caruana,[1] D. Abbadessa,[1] M. Usala,[2] G. Scaduto,[1] P. Colomba,[3] R. Alessandro[3]

[1]Urology Unit, P. Giaccone Hospital, University of Palermo; [2]Physiotherapy Unit, P. Giaccone Hospital, University of Palermo; [3]Department of Biology, P. Giaccone Hospital, University of Palermo, Italy

Introduzione

Abbiamo pensato di effettuare uno studio che indagasse circa la presenza delle citochine nell'albuginea di soggetti affetti da malattia di La Peyronie. Le citochine, in quanto responsabili della comunicazione intercellulare, potrebbero essere coinvolte nella patogenesi della malattia.[1,2] L'individuazione di una o più citochine responsabili potrebbe, infatti, risultare utile nel trattamento farmacologico grazie ai c.d. farmaci biologici, in grado di interferire con le citochine.[3]

Materiali e Metodi

Per lo studio sono stati reclutati tra gennaio 2009 e dicembre 2010 presso l'Ambulatorio di Chirurgia Andrologica del Policlinico di Palermo 20 soggetti affetti da malattia di La Peyronie (Figura 1) e 8 soggetti affetti da *recurvatum penis* congenito, questi ultimi adoperati come controllo. Come criteri di inclusione allo studio abbiamo considerato i criteri di operabilità con corporoplastica secondo Nesbit. I prelievi bioptici ottenuti dalle losanghe escisse in corso di intervento chirurgico sono stati utilizzati per saggiare l'espressione genica, attraverso Real-Time PCR, di citochine pro-fibrotiche e pro-infiammatorie. Inoltre sono stati sottoposti dopo fissazione ad esame istologico con la colorazione ematossilina-eosina.

Risultati e Discussione

L'esame istologico ha rilevato l'assenza di cellule infiammatorie in tutti i pazienti recensiti per lo studio. L'analisi dell'espressione dei geni codificanti per IL-4 (Interleuchina-4), IL-6 (Interleuchina-6), IL-13(Interleuchina-13), TGF-β1 (Trasforming Growth Factor-β1), IL-2 (Interleuchina-2), IL-10 (Interleuchina-10), TNF-α (Tumor Necrosis Factor-α) e IFN-γ (Interferone- γ) ha evidenziato in tutti i campioni un livello molto basso di trascritti e in alcuni casi indosabili (Figura 2). Inoltre i livelli dei trascritti delle citochine prese in esame sono risultati minori nei campioni provenienti dagli individui affetti da malaltia di La Peyronie rispetto ai controlli.

PZ	SEDE CURVATURA	DE	COITO	DURATA MALATTIA (mesi)	FATTORI DI RISCHIO
1	Laterale	no	impossibile	14	Ex fumatore, DM II, HCV+, TIA
2	Laterale	lieve	ok	36	DM II, sclerosi multipla
3	Dorsale	lieve	difficile	17	DM II, pregressa IPP
4	Ventrale	no	difficile	9	Ex fumatore, DM II
5	Dorsale	no	impossibile	12	ex fumatore, IMA
6	Laterale	moderato	impossibile	24	Ex fumatore, DM II
7	dorso-laterale	no	difficile	12	Ex fumatore, IA
8	dorso-laterale	no	difficile	19	Ex fumatore, IA
9	Dorsale	no	impossibile	24	IA, iperuricemia
10	Dorsale	lieve	ok	21	Ex fumatore, DM II
11	Ventrale	moderato	difficile	19	Ex fumatore, DM II
12	Dorsale	moderato	ok	30	Ex fumatore, DM II
13	Laterale	no	ok	23	Ex fumatore, DM II
14	Laterale	no	impossibile	12	Ex fumatore, DM II
15	Dorsale	lieve	impossibile	18	Ex fumatore, DM II
16	Dorsale	grave	difficile	24	Ex fumatore, IA
17	dorso-laterale	no	difficile	24	Ex fumatore, DM II
18	Laterale	grave	impossibile	10	Ex fumatore, IA
19	dorso-laterale	no	impossibile	15	Ex fumatore, DM II
20	Dorsale	moderato	difficile	24	Ex fumatore, IA

Figura 1. Caratteristiche dei pazienti affetti da Malattia di La Peyronie.

Correspondence: Carlo Pavone, Urology Unit, P. Giaccone Hospital, University of Palermo, via del Vespro 129, 90127 Palermo, Italy.
E-mail: uropa@unipa.it

Journal of Biological Research 2015; 88:5161

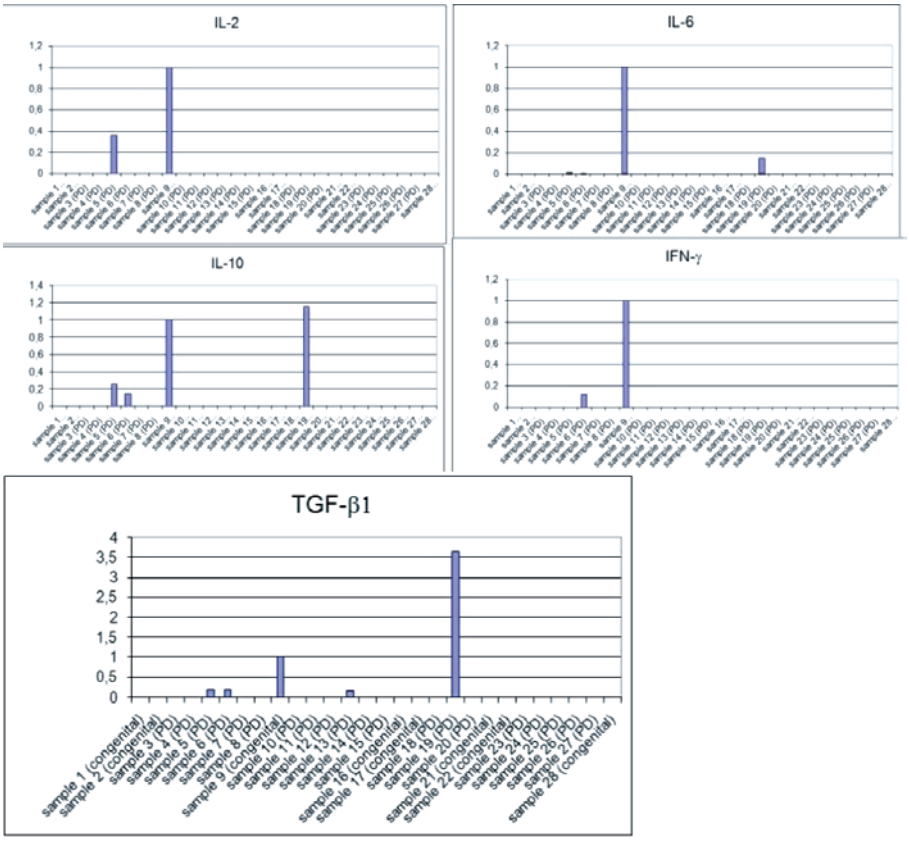

Figura 2. Livelli di trascritti delle citochine dosate.

Conclusioni

Alla luce dei risultati ottenuti, l'utilizzo di farmaci biologici (anticorpi) contro le citochine non sembra essere applicabile nella fase stabile della malattia di La Peyronie.

Bibliografia

1. Haag SM, Hauck EW, Eickelberg O, Szardening-Kirchner C, Diemer T, Weidner W. Investigation of the antifibrotic effect of IFN-gamma on fibroblasts in a cell culture model of Peyronie's disease. Eur Urol 2008;53:425-30.
2. Piao S, Choi MJ, Tumurbaatar M, Kim WJ, Jin HR, Shin SH, Tuvshintur B, Yin GN, Song JS, Kwon MH, Lee SJ, Han JY, Kim SJ, Ryu JK, Suh JK.Transforming growth factor (TGF)-β type I receptor kinase (ALK5) inhibitor alleviates profibrotic TGF-β1 responses in fibroblasts derived from Peyronie's plaque. J Sex Med 2010; 7:3385-95.
3. Zimmermann RP, Feil G, Bock C, Hoeltl L, Stenzl A. Significant alterations of serum cytokine levels in patients with Peyronie's disease. Int Braz J Urol. 2008;34:457-66.

Qualità della vita e livelli di cortisolo

M. Coco,[1] M. Blandini,[1] T. Maci,[2] M.C. Petralia,[1] S. Massimino,[1] V. Perciavalle,[1] V. Perciavalle[3]

[1]Physiology Unit, Department of Bio-Medical Sciences, University of Catania; [2]Department G.F. Ingrassia, University of Catania; [3]Department of Formative Process, University of Catania, Italy

Introduzione

Lo stress psicologico è da un lato una condizione che potenzia le capacità reattive dell'individuo di fronte a condizioni di allarme e dall'altro un fattore di rischio per lo sviluppo e la progressione di numerose malattie.[1] È opinione condivisa dalla comunità scientifica che l'attività fisica sia in grado di indurre cambiamenti fisiologici a largo spettro, influendo positivamente anche suli livelli di stress.[2] Scopo del presente lavoro è stato quello di valutare se ed in che modo una particolare tecnica di rilassamento, nota come tecnica di respirazione profonda (deep breathing), fosse in grado di produrre miglioramenti fisiologici sui valori di cortisolo, su un campione di soggetti volontari.

Metodi

Hanno preso parte allo studio 38 soggetti volontari sani; 19 hanno costituito il gruppo di soggetti sui quali studiare gli effetti della respirazione profonda (deep breathing) e 19 il gruppo di controllo. Tutti i soggetti hanno firmato il consenso informato preparato sulla base delle indicazioni del Comitato Etico della nostra Università. I livelli di stress psicologico sono stati misurati sulla base dei livelli di cortisolo salivare.[3-5] Il protocollo consisteva in 10 sedute di deep breating[6] della durata di un'ora e trenta minuti. Sono state eseguite le misurazioni dei livelli di cortisolo in tre tempi: la prima è stata all'inizio durante la prima sessione (I), la seconda durante la quinta sessione (II), la terza durante l'ultima sessione (III).

Risultati

I valori di cortisolo ottenuti durante la I sessione mostrano valori simili fra il gruppo di controllo ed il deep breathing group. La Figura 1 mostra a sinistra i valori di cortisolo misurati nei soggetti facenti parte del deep breathing group; è possibile osservare un miglioramento statisticamente significativo fra la I e la III sessione. La stessa figura mostra a destra i valori di cortisolo del gruppo di controllo che non mostrano modificazioni significative nelle diverse sedute.

Discussione e Conclusioni

I presenti risultati confermano quelli di Martarelli e collaboratori[6] che hanno osservato come il rilassamento indotto da respirazione diaframmatica aumenti lo stato di difesa antiossidante negli atleti dopo esercizio esaustivo, e che questi effetti si correlano con la concomitante diminuzione del cortisolo. I risultati ottenuti dal presente lavoro mostrano un miglioramento significativo dei valori di cortisolo nel gruppo che ha praticato deep breathing. È possibile ipotizzare che praticare con regolarità e costanza la tecnica di respirazione profonda possa essere un facile e utile strumento per una buona qualità della vita, agendo indirettamente sulla gestione delle tensioni- stress che la vita di tutti i giorni costringe a seguire.

Bibliografia

1. Muscatell KA, Eisenberger NI. a social neuroscience perspective on stress and health. Soc Personal Psychol Compass 2012;6:890-904.
2. Best John R. Effects of physical activity on children's executive function: Contributions of experimental research on aerobic exercise. Devl Rev 2010;30:331-51.

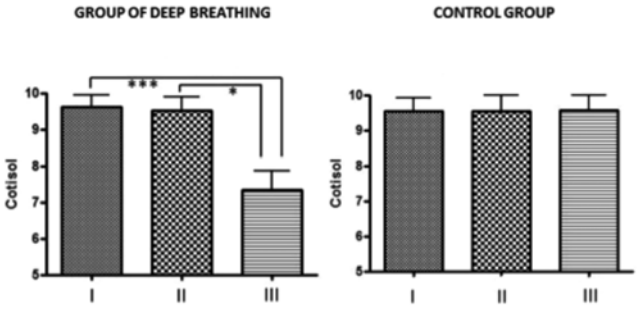

Figura 1. Livelli di cortisolo salivare misurato nelle diverse sessioni (I, II, e III) nei soggetti che praticavano deep breathing (a destra) e nei controlli.

Correspondence: Valentina Perciavalle, Department of Formative Process, University of Catania, via Ofelia, 95124 Catania, Italy.
E-mail: valentinaperciavalle@unict.it

3. Hellhammer DH, Wust S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology 2009;34: 163-71.
4. Cacioppo JT. Somatic responses to psychological stress: the reactivity hypothesis. In: Adair JG, Belanger D, Dion KL, eds. Advances in psychological science. Hove, UK: Psychology Press; 1998. pp 87-109.
5. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychobiological research: an overview. Neuropsychobiology 1989; 22:150-69.
6. Martarelli D, Cocchioni M, Scuri S, Pompei P. Diaphragmatic breathing reduces exercise-induced oxidative stress. Evid Based Complement Altern Med 2011;2011:932430.

Oxidative stress markers associated with middle distance running performance

P. Proia, M. Giaccone, V. Contrò, A. Bianco, M. Traina, A. Palma

Department of Legal, Social and Sports Sciences, University of Palermo, Italy

The aim of this study was to understand the mechanism underlying the physiological adaptation of a kind purely aerobic workout. Particular attention has also been given to the analysis of oxidative stress by detecting some enzymatic blood parameters. We investigate the effect of 2 months of training on middle distance running (800 meters and 1500 meters) agonistic athletes; nine active specifically trained males of mean (±SD) age 19,9 years. The physiological characteristics of middle runners are different from those sprinters and long distance runners because include a variety of aerobic and anaerobic capabilities. Two weeks prior to the 2 months period of exercise, subjects were tested for VO_{2max} during a graded, treadmill test with the Cosmed FitMate metabolic device (Cosmed, Italy). Antropometric parameters were detected used a Bioimpedance analyzer (BF 302 Ormon BIA). A blood sample was collected in the morning and were collected by a clinical specialized center to analyze: triglycerides, total cholesterol, G.O.T. and G.P.T. transaminase, γ-GT, CPK and CK-MB, as well as lipid profile. All these tests were performed before and after the two months of training period. The results obtained suggest that the endurance training, as it is high oxygen consumption, should increase reactive oxygen species (ROS), but it has been shown that exercise leads to increased activation of antioxidant defenses. Infact, serum levels of gamma-glutamyltransferase (GGT) enzyme, which plays a key role in the metabolism of extracellular reduced glutathione[1] was not increased. However, a classic cardiac biomarkers,[2] CK-MB as well as total CK was analyzed and while the total CK after two months of training increased, the CK-MB isoform decrease, in a significant statistical way. Even the emathological parameters were analyzed and there were the variations overall on neutrophils and monocytes value. These two cell type are involved in the infection respons. An antropometric parameter that changed after two months of training, was the weight. Infact after statistical analysis, the P value was <0.0001, considered extremely significant (59.3±5.4 kg before training; 58.1±5.2 kg after training). Finally, put together all the results, we can say that middle distance runners are subject at not high oxidative and biological stress; infact there were no change in γ-GT value, that usually is involved in the oxidative stress as well as a decrease of CK-MB value, that usually associated with cardiac injury. These are preliminary results that need to confirm with other studies using a larger sample of subjects or comparing with the runners that are involved in a different running distance (sprinters or long distance runners).

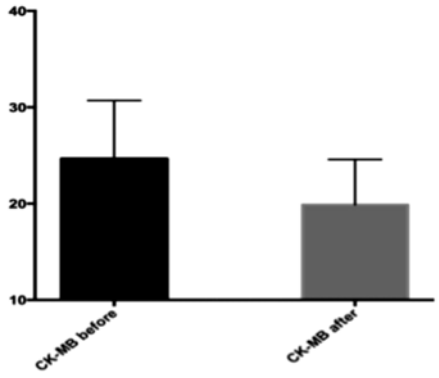

Figure 1. Changes in CK-Mb value after two months of training.

References

1. Danielsson J, Kangastupa P, Laatikainen T, et al. Individual and joint impacts of ethanol use, bmi, age and gender on serum gamma-glutamyltransferase levels in healthy volunteers. Int J Mol Sci 2013;14:11929-41.
2. Nursalim A, Suryaatmadja M, Panggabean M. Potential clinical application of novel cardiac biomarkers for acute myocardial infarction. Acta Med Indones 2013;45:240-50.

Correspondence: Patrizia Proia, Department of Legal, Social and Sports Sciences, University of Palermo, via Duse 1, Palermo, Italy.
E-mail: patrizia.proia@unipa.it

Journal of Biological Research 2015; 88:5161

La comunità Inuit di Igloolik (Canada) nella seconda metà del Novecento: una ricerca pionieristica di citogenetica per raccontare la storia di un popolo

D. Minaldi,[1] B. Chiarelli,[2] R. Boano,[1] E. Rabino Massa[1]

[1]Department of Life Sciences and Biology of Systems, University of Torino ; [2]Department of Evolutionary Biology, University of Florence, Italy

Negli anni 1971-1972 nell'ambito dell'*International Biological Programme: Human Adaptability Project,* l'UNESCO promosse un progetto di ricerca sull'insediamento esquimese di Igloolik (Canada). Il programma di ricerca, coordinato dal Prof. Brunetto Chiarelli, già ordinario di Antropologia presso l'Università degli Studi di Firenze, aveva come obiettivo lo studio delle caratteristiche genetiche della popolazione eschimese a confronto con le caratteristiche delle popolazioni urbanizzate. Lo spunto per questo studio nasceva dall'ipotesi che le popolazioni isolate, non esposte ai vari agenti mutageni del mondo industrializzato, avrebbero dovuto presentare una percentuale molto bassa di aberrazioni cromosomiche rispetto alle altre popolazioni. Gli Eschimesi o meglio gli *Inuit*, per le loro caratteristiche di popolazione adattata ad un ambiente estremo e isolata biologicamente, si rivelarono un campione di studio adatto per sperimentare le tecniche della moderna citogenetica. Agli inizi degli anni Settanta la disciplina aveva già conosciuto la grande rivoluzione metodologica rappresentata dalla tecnica del bandeggio che consente di produrre bande orizzontali di differente intensità di colorazione su tutti i cromosomi del corredo rendendo possibile così una facile identificazione e un preciso accoppiamento. Lo studio condotto negli anni Settanta sugli abitanti di Igloolik rappresentò la prima applicazione delle tecniche di bandeggiamento dei cromosomi su popolazioni artiche. Il nostro contributo ha lo scopo di ripercorrere la storia di questo studio pionieristico e del contesto storico-geografico che lo ispirò alla luce anche delle nuove metodologie di indagine, tuttora in continuo perfezionamento. Vogliamo, inoltre, sottolineare che gli strumenti e le tecniche del passato possono rappresentare un patrimonio di conoscenze con valenze museali quando sono associati alle testimonianze materiali del popolo studiato.

Correspondence: Donatella Minaldi, Department of Life Sciences and Biology of Systems, University of Torino, via Accademia Albertina 13, 10123 Torino, Italy.
E-mail: donatella.minaldi@unito.it

Journal of Biological Research 2015; 88:5161

Procianidina b2 ed (–)-Epicatechina presenti nelle mele: studio preliminare sul loro effetto sui meccanismi di aggregazione e di inibizione di proteine amiloidi

G.C. Rappa,[1,2] V. Guarrasi,[1] S. Vilasi,[1] M.A. Germanà,[2] P.L. San Biagio[1]

[1]Institute of Biophysics, National Research Council, Palermo; [2]Department of Agricultural and Forestry Sciences, University of Palermo, Italy

I polifenoli estratti da diversi frutti, come mele, pompelmi, arance, limoni o melograni, sono noti per le loro azioni benefiche sulla salute dell'uomo, per la loro attività antiossidante,[1] antitumorale[2] e anti-infiammatoria.[3] È stato, inoltre, riscontrato un loro effetto neuroprotettivo e anti-amiloidogenico *in vitro*.[4,5] Studi recenti hanno infatti dimostrato che alcuni polifenoli, come l'acido gallico, l'epicatechina gallate e l'epigallocatechina gallate esercitano un'azione neuroprotettiva nei confronti della tossicità indotta dalla proteina β- amiloide (peptide Aβ), coinvolta nella patologia dell'Alzheimer.[4] In particolare è stato dimostrato come polifenoli estratti dalle mele, con alto contenuto di procianidine, siano in grado di inibire *in vitro* l'aggregazione del peptide Aβ.[6] La malattia di Alzheimer (AD) è la forma più comune di demenza neurodegenerativa invalidante ad esordio prevalentemente senile. Colpisce le più alte funzioni cerebrali come la memoria e le funzioni cognitive e ciò si ripercuote sulle capacità intersociali dei malati di tale patologia causando stati confusionali, cambiamenti di umore e disorientamento spazio-temporale. Osservazioni eseguite tramite impiego di tecniche di immagine, indicano che i pazienti affetti da AD mostrano, a livello neurologico macroscopico, una diminuzione del peso e del volume cerebrale per atrofia corticale, con allargamento dei solchi e corrispondente appiattimento delle circonvoluzioni. Nei pazienti con AD si osservano deposizioni a livello dell'ippocampo e della corteccia cerebrale chiamate placche senili, costituite da aggregati proteici di un peptide di 40-42 amminoacidi, il peptide β-amiloide. Il peptide Aβ deriva da uno specifico *pathway* proteolitico di una glicoproteina di transmembrana ubiquitariamente espressa, nota come proteina precursore dell'amiloide le cui funzioni non sono ancora conosciute, anche se recenti studi suggeriscono una sua implicazione nella formazione delle sinapsi, nella plasticità neuronale e nell'esporto di ferro. L'accumulo del peptide Aβ è ipotizzato come inizio di una cascata patogenetica che eventualmente porta alla malattia.[7] In condizioni fisiologiche il rapporto tra Aβ-42 e Aβ-40 è circa 1:10. In letteratura, l'aggregazione del peptide amiloide è descritta secondo una tipica cinetica di nucleazione-polimerizzazione, in cui ognuna delle fasi è caratterizzata da specifici intermedi strutturali che presentano diverse dimensioni, morfologie e potenziale citotossico.[8]

Il presente lavoro è finalizzato allo studio dell'effetto di alcuni composti naturali, quali le procianidine, sull'inibizione del processo di formazione di fibre amiloidi *in vitro*.

Mediante cromatografia liquida (HPLC-UV/Vis) si è provveduto inizialmente all'identificazione ed alla quantificazione di polifenoli in mele della cultivar *Gala*, coltivate in Sicilia in regime di agricoltura biologica. Studi bibliografici individuano cinque principali gruppi polifenolici in diverse varietà di mele: acidi idrossicinnamici, procianidine, antocianine, flavonoli e diidrocalconi. Le procianidine sono i maggiori componenti del profilo polifenolico, presenti per il 59,7% nella buccia e per il 55,7% nella polpa.[9] Dopo aver verificato la presenza di Procianidina B2 e del monomero (–)-Epicatechina tra le procianidine presenti in queste mele, è stato valutato mediante spettroscopia di fluorescenza (*Test della Tioflavina T*) il loro effetto sulle proprietà di aggregazione di una proteina modello, la k-caseina, che, analogamente al peptide Aβ, forma fibre amiloidi a partire da un monomero intrinsecamente disordinato che contribuisce al core fibrillare con un *double strand* β. Le misure di fluorescenza mostrano che sia il dimero che il monomero, se pur in misura diversa, inibiscono in maniera dose-dipendente l'aggregazione della k-caseina. Misure di dicroismo circolare (CD) hanno anche permesso di valutare la variazione della struttura secondaria di aggregati formati in assenza ed in presenza di Procianidina B2 ed (–)-Epicatechina. Dagli spettri CD risulta che l'(–)-Epicatechina (65 μg/mL) non influenza la conversione strutturale della proteina a foglietti β ordinati, caratteristici delle specie amiloidi. Infatti dopo incubazione a 37°C per 44 ore si assiste allo *shift* del minimo CD da 205nm (valore di pre-incubazione) verso lunghezze d'onda maggiori, analogamente a quanto riscontrato nel campione controllo (k-caseina nativa). I risultati ottenuti costituiscono la base metodologica-sperimentale per la successiva analisi dell'effetto di tali polifenoli sull'aggregazione e tossicità del peptide Aβ coinvolto nel morbo di Alzheimer e per l'indagine sul loro meccanismo di azione ai fini di un potenziale impiego terapeutico.

Correspondence: Valeria Guarrasi, Institute of Biophysics, National Research Council, via Ugo La Malfa 153, Palermo, Italy.
E-mail: valeria.guarrasi@pa.ibf.cnr.it

Journal of Biological Research 2015; 88:5161

Bibliografia

1. Leontowicz H., Gorinstein S., Lojek A., Leontowicz M., Ciz M., Soliva-Fortuny R.C., Park Y-S., Jung S-T., Trakhtenberg S. and Martin-Belloso O. Comparative content of some bioactive compounds in apples, peaches and pears and their influence on lipids and antioxidant capacity in rats. Journal of Nutritional Biochemistry, 2002, vol. 13, pp. 603-10.

2. Femia A.P., Caderni G., Vignali F., Salvadori M., Giannini A., Biggeri A., Gee J., Przybylska K., Cheynier V., Dolara P. Effect of polyphenolic extracts from red wine and 4-OH- coumaric acid on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats. European Journal of Nutrition, 2005, vol. 44, pp. 79-84.
3. Terra X., Palozza P., Fernandez-Larrea J., Ardevol A., Blade C., Pujadas G., Salvado J., Arola L. and Blay M.T. Procyanidin dimer B1 and trimer C1 impair inflammatory response signalling in human monocytes. Free Radical Research, 2011, vol. 45, pp. 611-9.
4. Bastianetto S., Yao Z.X., Papadopoulos V. and Quirion R. Neuroprotective effects of green and black teas and their catechin gallate esters against β-amyloid-induced toxicity. European Journal of Nutrition, 2006, vol. 23, pp 55-64.
5. Ono K., Yoshiike Y., Takashima A., Hasegawa K., Naiki H. and Yamada M. Potent anti- amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease. Journal of Neurochemistry, 2003, vol. 87, pp 172-8.
6. Toda T., Sunagawa T., Kanda T., Tagashira M., Shirasawa T. and Shimizu T.. Apple Procyanidins Suppress Amyloid β-protein Aggregation. Biochemistry Research International, 2011, pp 1-8.
7. Hardy J.A., Higgins G.A. Alzheimer's disease: the amyloid cascade hypothesys. Science, 1992, vol. 256, pp 184-5.
8. Carrotta R., Canale C., Diaspro A., Trapani A., San Biagio P.L., Bulone D. Inhibiting effect of s1-casein on Aβ1-40 fibrillogenesis. Biochimica et Biophysica Acta, 2012, vol. 1820, pp 124-32.
9. Tsao R., Yang R., Yang J.C. and Zhu H. Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). Journal of Agriculture Food Chemistry, 2003, vol. 51, pp 6347-53.

Comparative analysis of Hsp10 and Hsp90 in large bowel healthy mucosa and adenocarcinomas

F. Rappa,[1,2] M. Lo Bello,[1] C. Caruso Bavisotto,[1] A. Marino Gammazza,[1,2] C. Campanella,[1,2] S. David,[1] C. Sciumè,[3] F. Zarcone,[3] G. Geraci,[3] G. Modica,[3] F. Carini,[1] G. Tomasello,[2,3] M. Bellavia,[2] M.L. Uzzo,[4] G. Spatola,[4] G. Bonaventura,[4] A. Leone,[4] P. Damiani,[5] F. Buccheri,[1,2] A. Gerbino,[4] F. Farina,[1] F. Cappello[1,2]

[1]Department of Experimental Biomedicine and Clinical Neuroscience, Section of Human Anatomy, University of Palermo; [2]Euro-Mediterranean Institute of Science and Technology, Palermo; [3]Department of Surgical, Oncological and Stomatological Sciences, University of Palermo; [4]Department of Experimental Biomedicine and Clinical Neuroscience, Section of Histology and Embryology, University of Palermo; [5]Department of Internal and Specialty Medicine, University of Palermo, Italy

Introduction

Heat shock proteins (Hsps) are an important class of molecules with various functions. Their classic role is to assist other proteins in folding and re-folding and, when proteins are defective or irreversibly misfolded, to drive their degradation. For this reason, some Hsps are also named molecular chaperones. During evolution, this class of proteins has also acquired *extrachaperoning* roles such as participation in immune system regulation, cell differentiation, programmed cell death and carcinogenesis. Hsp10 is a partner of Hsp60 in the Hsp60/10 folding machine, but numerous scientific studies have shown that Hsp10 may also play other roles. In fact, Hsp10 seems to have an immunomodulatory activity and a role in tumor progression. Hsp90 regulates late-stage maturation, activation and stability of a range of *client* proteins, such as HER2, EGFR and BRAF, some of which are involved in signal transduction and other key pathways important for malignancy in several cancers, including large bowel carcinomas. The aim of the present study was to evaluate levels and expression of Hsp10 and Hsp90 in a series of samples of large bowel mucosa obtained from healthy controls and patients with adenocarcinomas.

Correspondence: Francesca Rappa, Department of Experimental Biomedicine and Clinical Neuroscience, Section of Human Anatomy, University of Palermo, via del Vespro 129, 90127 Palermo, Italy.
E-mail: francyrappa@hotmail.com

Journal of Biological Research 2015; 88:5161

Materials an Methods

Twenty samples of large bowel human mucosa from healthy control subjects and twenty samples of large bowel adenocarcinomas with moderate grade of differentiation, were obtained from the DICHIRONS Department of the University of Palermo, Italy. RT-PCR and Western Blotting analyses were performed on these samples in order to study gene and protein expression of Hsp10 and Hsp90 (both Hsp90 and Hsp90 isoforms). Moreover, an immunohistochemical study for Hsp10 and Hsp90 was performed to evaluate the localization of these proteins in both the epithelium and the lamina propria.

Results

RT-PCR analysis showed a higher gene expression of Hsp10 and Hsp90 in adenocarcinoma samples compared to healthy mucosa. The Western Blotting analysis confirmed a greater amount of Hsp10 and Hsp90 proteins in the samples of adenocarcinoma of large bowel compared to healthy mucosa. Finally, levels of Hsp10 were higher in adenocarcinoma compared to normal mucosa in both the epithelium and in the lamina propria, as revealed by immunohistochemistry. By contrast, Hsp90 levels were not significantly different in the epithelium, while they were higher in the lamina propria of adenocarcinoma samples compared to normal mucosa.

Conclusions

These data suggest that Hsp10 and Hsp90 may be involved in the carcinogenesis of the large bowel by different molecular mechanisms.

Nutrizione, obesità e sindrome metabolica in un campione di anziani della Val Cenischia

M. Rinaldi,[1] G. Graffi,[2] E. Rabino Massa[1]

[1]Laboratory of Anthropology, Department of Life Sciences and Biology of Systems, University of Turin; [2]Local Health Service ASL TO3, Susa (TO)

In un campione di 189 persone con più di 60 anni appartenenti alle comunità della Val Cenischia (Piemonte) e originari della valle da almeno tre generazioni è stata valutata la prevalenza di sindrome metabolica secondo i criteri del NCEP ATPIII.[1] A questo fine ad ogni partecipante è stato effettuato un prelievo venoso a digiuno per la determinazione della glicemia, della trigliceridemia e della colesterolemia totale e HDL, è stata misurata la pressione arteriosa, sono state rilevate alcune misure antropometriche (peso, altezza e circonferenza addominale) ed è stata somministrata un'intervista standardizzata volta ad indagare le abitudini alimentari, il livello di attività fisica ed altri aspetti dello stile di vita.

Nel campione, composto da 104 donne (età media: 73,6±8,4 anni) e 85 uomini (età media: 73,0±7,4 anni) la sindrome metabolica ha una prevalenza del 42,1% (F=43,2%; M=40,8%). Lo stato di sovrappeso (definito da un BMI compreso tra 24,9 e 29,9 kg/mq) ha una prevalenza del 39,7% (F=40,2%; M=39,1%), mentre l'obesità (definita da un BMI≥30,0 kg/mq) del 23,8% (F=22,5%; M=25,3%).

Dall'analisi dei dati clinici e dei dati nutrizionali è emersa una associazione significativa tra l'apporto nutrizionale della prima colazione e uno stato di obesità/sovrappeso. In particolare è stata riscontrata la presenza di una correlazione negativa tra il valore energetico della prima colazione e il BMI (R=-0,227, P=0,002). Le donne che consumano una colazione non adeguata (cioè inferiore al 15,0% dell'apporto energetico quotidiano) hanno un BMI medio significativamente superiore rispetto a coloro che consumano una colazione adeguata (27,6 *vs* 25,6; T=2,669, DF=102, P=0,009) e lo stesso avviene per gli uomini (31,0 *vs* 26,7; T=3,298, DF=83, P=0,001).

Inoltre, è emersa un'associazione significativa anche tra assunzione di quantità eccessive di alcool (>20 g/dì per le donne e >40 g/dì per gli uomini) e obesità addominale (definita da una circonferenza addominale >88 cm per le donne e >102 cm per gli uomini) in entrambi i sessi (donne: χ^2=4,989; DF=1; p=0,025; O.R.=2,706 [IC95%: 1,115 - 6,569]; uomini: χ^2=7,167; DF=1; P=0,007; O.R.=3,525 [IC95%: 1,363-9,119]).

Questo lavoro fa parte di un progetto di ricerca più ampio svolto in collaborazione con il dipartimento di Neuroscienze dell'Università di Torino e con il Dipartimento di Scienze Biologiche, Geologiche e Ambientali dell'Università di Bologna volto a studiare il decadimento cognitivo legato all'invecchiamento in relazione a fattori di rischio cardiovascolari di tipo clinico, genetico e comportamentale e ha ricevuto l'approvazione del Comitato di Bioetica dell'Università di Torino.

Bibliografia

1. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). JAMA 2001;285:2486-97.

Correspondence: Marianna Rinaldi, Laboratory of Anthropology, Department of Life Sciences and Biology of Systems, University of Turin, via Accademia Albertina 13, 10123 Torino.
E-mail: marianna.rinaldi@unito.it

Journal of Biological Research 2015; 88:5161

Plasma serotonin in horses: comparison between two different management conditions

D. Alberghina, C. Giannetto, M. Rizzo, M. Panzera, G. Piccione

Department of Veterinary Sciences, University of Messina, Italy

Recent reports of new and important roles for serotonin in the periphery have served to increase interest in circulating serotonin (5-HT). The much smaller pool of free (extraplatelet) plasma 5- HT is accessible to sites of action and receptors, and may be important in many processes. Assessing this extraplatelet plasma pool could be very difficult also because many factors could influence 5-HT levels.[1] Horses kept in stalls are deprived of opportunities for social interactions and the performance of natural behaviors is limited. The hypothesis of this study was that stalling horses results in a negative effect on their welfare. As marker of poor welfare we evaluate plasma 5-HT and its precursor tryptophan (TRP) in 14 adult horses heterogeneous for sex, breed and age (13±7 years). In previous studies lower levels of plasma 5-HT were found in horses with cribbing behaviour[2] and in subjects feed with high levels in concentrates.[3] Horses of this study were divided in two groups: Stall (S) Group and Pasture (P) Group. Group S (n.6 horses) was maintained in individual box under a natural photoperiod (sunrise at 06:06, sunset at 18:49) and natural indoor temperature (19-21°C) from the day before the experiment to the afternoon of follow day. Horses were fasted overnights (12-14 hours) and then feed with hay that was provided at 08:30 and 12:30, water was available *ad libitum*. Group P (n.8 horses) was maintained at the same condition of Group S until 8:30 then it was transferred from box stalls to pasture. Blood samples were obtained from the jugular vein at 08:00, 12:00 and 16:00 and collected into EDTA-containing tubes. Within 30 minutes from the venipuncture, sample tubes were centrifuged at 1350 x g for 10 minutes to obtain the fraction defined as platelet poor plasma. One hundred µL of plasma were then supplemented with an equal volume of an internal standard represented by N-methylserotonin and treated with 100 µL of a precipitating reagent to ensure protein removal. Samples were the vortex-mixed for 30 seconds, allow to stand for 10 minutes at 4°C and centrifuged in a top-bench centrifuge at the maximal speed. The resulting clear supernatants were stored at -20°C and analysed within one week for the HPLC quantification of 5-HT and TRP according to protocols earlier described.[4] All the results obtained were expressed as mean values±standard deviation (SD). One-way repeated measure analysis of variance (ANOVA) was performed to determine the statistically significance and Bonferroni's test was applied as post hoc comparison test. Mann Whitney test was used to compare differences between groups. The data were analysed using the software STATISTICA 8 (Stat Soft Inc.). Results for 5-HT and TRP are shown on Table 1 and Figure 1 respectively. The influence of time evaluate by ANOVA was significant in both Groups ($P<0.001$) with levels significantly higher at 12:00 and at 16:00 compared to levels at 08:00. 5-HT levels were significantly higher in Group P compared to Group S at 12:00 ($P<0.01$) and 16:00 ($P<0.001$). Also TRP levels were significantly influenced by time in both Groups ($P<0.001$) with higher levels at 12:00 and at 16:00 compared to levels at 08:00. No difference between Groups were found for TRP concentrations. The pattern of 5-HT and TRP levels confirmed previous results on equine daily rhythms for these parameters.[5] The lower levels of 5-HT measured at 12:00 and at 16:00 in Group S could indicate that factors as absence of exercise and isolation could influence 5-HT levels. Regardless of this, we recognize that, in addition to differences in exercise and social interaction there are confounding factors between treatment groups including nutrition rate and exposure to sunlight. However, these same confounding factors would be present in any operation where a decision has to be made as to whether to stall horses or provide access to pasture. In conclusion obtained results in the present study showed the modulation of plasma 5-HT by two different management conditions. Our suggestion is to improve the knowledge about factors that can increase plasma equine 5-HT levels in order to guarantee the animal welfare.

Correspondence: Giuseppe Piccione, Department of Veterinary Sciences, University of Messina, Polo Universitario dell'Annunziata, 98168 Messina, Italy.
E-mail gpiccione@unime.it

Journal of Biological Research 2015; 88:5161

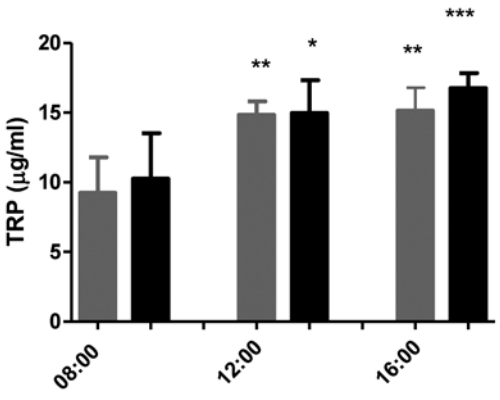

Figure 1. Patterns of mean values (±standard deviation) of plasma tryptophan in Group S (grey bar) and in Group P (dark bar) from 08:00 to 16:00.

Tabella 1. Patterns of mean values (±standard deviation) of plasma 5-HT (ng/mL) from 08:00 to 16:00 in horses maintained in individual box (08:00 both groups, 12:00 and 16:00 Group S) and at pasture (Group P: 12:00 and 16:00).

Time	Experimental condition			ANOVA
	08:00	12:00	16:00	
5-HT ng/mL (Group S)	29.4±10.0	110.9±27.1^{c}	121.7±31.2^{c}	F2,21=33.66; P<0.001
5-HT ng/mL (Group P)	23.0±4.78	42.04±6.8^{b*}	68.13±11.2^{c**}	F2,15=47.81; P<0.001

Bonferroni post-hoc comparison: *vs* 08:00 ^{c}P<0.001; ^{b}P<0.01. Mann-Whitney test: *vs* Group S * P<0.01; **P<0.001.

References

1. Brand T, Anderson GM. The Measurement of Platelet-Poor Plasma Serotonin: A Systematic Review of Prior Reports and Recommendations for Improved Analysis. Clin Chem 2011;57;1376-86.
2. Lebelt D, Zanella AJ, Unshelm J. Physiological correlates associated with cribbing behaviour in horses: changes in thermal threshold, heart rate, plasma beta-endorphin and serotonin. Equine Vet J Suppl 1998;27:21-7.
3. Alberghina D, Giannetto C, Visser EK, Ellis AD. Effect of diet on plasma tryptophan and serotonin in trained mares and geldings. Vet Rec 2010;166:133-6.
4. Alberghina D, Amorini AM, Lazzarino G. Modulation of peripheral markers of the serotoninergic system in healthy horses. Res Vet Sci 2011;90:392-5.
5. Piccione G, Assenza A, Fazio F, et al. Central fatigue and nycthemeral change of serum tryptophan and serotonin in the athletic horse. J Circadian Rhythms 2005;3:6.

Evaluation of some oxidative stress markers in *ovis aries* during different experimental conditions

V. Monteverde,[1] M. Rizzo,[2] D. Alberghina,[2] F. Fazio,[2] E. Giudice,[2] G. Piccione[2]

[1]Institute for Experimental Veterinary Medicine of Sicily, Palermo; [2]Department of Veterinary Sciences, University of Messina, Italy

Oxidative stress can be regarded as an imbalance between prooxidant/free radical production and opposing antioxidant defenses. There is growing evidence that oxidative stress (OS) significantly impairs organic function and plays a major role in the aetiology and pathogenesis of several metabolic diseases in veterinary medicine.[1] In view of this, we evaluate some oxidative stress markers in ten Comisana ewes (3 years old, clinically healthy) at different experimental conditions: shearing, road transportation, different reproductive status. Moreover we evaluate daily rhythms of oxidative stress markers. On each subject, blood samples were collected by jugular venipuncture to assess oxidative stress markers during: shearing (before, after shearing and 8h, 1d, 2d, 3d, 4d, 5d post- shearing); road transportation for 6 h over a distance about 490 km with an average speed of 80 km/h (before, after transportation and 8, 24, 48 h post-transport); different reproductive status (days 1, 40 and 200 of lactation and during dry period); and circadian rhythm under natural photoperiod -sunrise 5:10, sunset 20:45- (every 3 h over a 24 h period, starting at 8:00 on day 1 and finishing at 8:00 on day 2). All blood samples were collected into vacutainer tubes with no additive and were centrifuged at 3000×g for 20 min. The sera obtained were immediately analyzed by means of a UV spectrophotometer (Slim SEAC, Firenze, Italy) for the assessment of dROMs, Oxy-adsorbent and SHp. These techniques are based on the *spin traps* system, molecules which react with free radicals, creating complexes revealed by spectrophotometry. During milking and dry period was also evaluate daily rhythm of oxidative stress markers (mean level, amplitude, acrophase and robustness) applying a trigonometric statistical model on mean values. All the results obtained were expressed as mean values±standard deviation (SD). Data were normally distributed ($P<0.05$, Kolmogorov-Smirnov test). One-way repeated measure analysis of variance (ANOVA) was performed to determine the statistically significance and Bonferroni's test was applied as post hoc comparison test. The data were analyzed using the software STATISTICA 8 (Stat Soft Inc.).

Correspondence: Francesco Fazio, Department of Veterinary Sciences, University of Messina, Polo Universitario dell'Annunziata, 98168 Messina, Italy.
E-mail ffazio@unime.it

Journal of Biological Research 2015; 88:5161

The lowest values of dROMs observed before shearing and a their subsequent increase, could be due to the energy deficiency that occurs in ewes after shearing. Ambient temperature, relative humidity and shearing can influence thermoregulatory mechanisms and the productive performance and welfare of ewes. The high values of oxy-adsorbent and SHp values further demonstrate the compensatory response of the organism to the increase of free radicals (dROMs) inducing oxidative stress after shearing (Table 1). Our results indicate that shearing causes a change in the ewes homeostatic balance that leads to oxidative stress. The road transportation in ewes appears to have an influence on the increase of catabolic reactions that leads to the onset of oxidative stress. The results of our study (Table 1) showed an increase of dROMs, Oxy-adsorbent and SHp values in ewes after the road transportation in comparison with basal level ($P<0.05$). The pattern of dROMs, characterized by a significant increase during post transport respect to, is in agreement with other researchers.[2] The increase of dROMs after 48 h rest time corresponds to approximately 95.85% and represents the energetic deficiency that occurs in ewes during transport period. The high values of Oxy-adsorbent and SHp during rest time further demonstrated the compensatory response of the organism to the increase of dROMs inducing oxidative stress successively to road transportation. During the different reproductive periods, lowest values of dROMs at the start of experimental period could be due to the energetic deficiency which occurs in ewes during the last period of pregnancy. The significant increase of dROMs on days 40 and 200 of lactation and during dry period compared to values obtained on day 1 of lactation shows high oxidative processes which occur during lactation in ewes. The pattern of dROMs characterized by low values at the beginning of lactation and by a significant increase at the mid-point of lactation was previously observed in lactating ewes.[3,4] The high values of Oxy-adsorbent and SHp at the end of lactation document the compensative response of the organism to oxidative stress. Oxidative processes increased ($P<0.05$) at the end of milking period (Figure 1), together with a compensative response of the organism to this stress and suggest the important role of oxidative status in dairy ewes. Our result showed that the high energetic requirements of milking period in ewes are directly proportional to free radicals formation and oxidative stress. The trigonometric statistical model of the single cosinor procedure indicated the existence of daily rhythm of dROMs, Oxy-ads and SHp in ewes with a nocturnal acrophase (dROMs=21:00; Oxy-ads.=21:30; SHp=21:15). All three markers showed a rhythm robustness above significance line (dROMs=81.5%; Oxy-ads.=79.1%; SHp=81.70%). In conclusion we can claim that there is a synergism between oxidative stress markers and the circadian rhythm of anti-oxidant power in ewes. The right zootechnics management and the formulation of ration that totally satisfy the requirements of the lactating subjects, seem o be essential in order to guarantee the productive status and the animal welfare.

Table 1. Patterns of mean values (±SD) of dROMs, Oxy-ads. and SHp and statistical significance changes about sampling time from pre-treatments.

Sampling time	Parameters					
	dROMs (UCARR)		Oxy-ads. (µmol/l)		SHp (µmol/l)	
	Shearing	Transport	Shearing	Transport	Shearing	Transport
before	31.80 ± 2.20	31.20 ± 2.03	1.930 ± 0.04	2.015 ± 0.14	102.70 ± 3.82	112.60 ± 2.58
after	33.80 ± 2.05	34.90 ± 2.09	1.939 ± 0.06	2.158 ± 0.18	104.80 ± 3.04	114.50 ± 2.25
after 8h	34.60 ± 1.93	36.40 ± 2.50■	1.941 ± 0.05	2.289 ± 0.18■	105.70 ± 5.06	125.70 ± 1.96■
after 1d	36.00 ± 1.74	48.10 ± 3.01■	1.957 ± 0.04	2.732 ± 0.16■	107.60 ± 3.32	146.30 ± 2.22■
after 2d	42.00 ± 1.49■	62.00 ± 2.94■	2.035 ± 0.04	2.892 ± 0.13■	117.80 ± 3.55	169.10 ± 2.95■
after 3d	45.60 ± 1.81■▲		2.179 ± 0.06■		156.60 ± 4.76■▲	
after 4d	55.00 ± 1.91■▲		2.335 ± 0.08■▲		210.60 ± 6.35■▲	
after 5d	70.20 ± 2.42■▲●		2.882 ± 0.08■▲		343.60 ± 4.72■▲	

Significance (P<0. 05): ■*vs* before; ▲*vs* after, after 8 h and after 1d; ●*vs* after 4d.

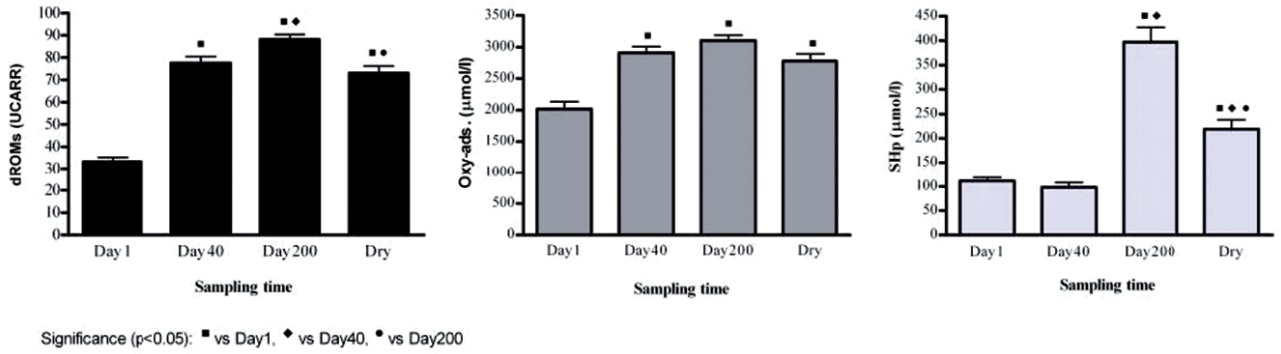

Figure 1. Mean values with relative standard deviations and statistical significance of daily patterns of dROMs, Oxy-ads. and SHp observed during different reproductive status.

References

1. Castillo Rodríguez C, Wittwer Menge F, Cerón JJ. Oxidative Stress in Veterinary Medicine. Veterinary Medicine International. 2011;2011:812086.
2. Halliwell B. Vitamin C: an antioxidant or pro-oxidant in vivo. Free Radical Res 1996;25:439-54.
3. Piccione G, Borruso M, Fazio F, et al. Oxidative stress evaluation during milking period in the ewes. J Appl Anim Res 2006;29:109-12.
4. Piccione G, Borruso M, Giannetto C, et al. Assessment of oxidative stress in dry and lactating cows. Acta Agr Scan A-An 2007;57:101-4.

Mechanical deflection of macrovibrissae induces in rats activation of trigeminal mesencephalic nucleus neurons

A. Russo,[1] S. Stanzani,[1] R. Pellitteri,[2] M.A. Caria,[3] O. Mameli[3]

[1]Physiology Section, Department of Biomedical Sciences, Catania University; [2]Institute of Neurological Sciences-National Research Council, Catania; [3]Human Physiology Division, Department of Clinical and Experimental Medicine, University of Sassari, Italy

The sensory system of rodent vibrissae has been extensively analysed to understand how these animals may successfully explore the nearby environment, detect objects and orient their behaviour using the macrovibrissae. Recent data[1] showed that besides the Gasser's ganglia neurons also the trigeminal mesencephalic nucleus (Me5) appears significantly involved in the sensory perception of the whisker pad structures. In fact: i) Tracer injection into mystacial pad of the rats significantly labelled the Me5 neurons and ii) electrophysiological experiments showed that Me5 neurons are responsive to spontaneous movement of the macrovibrissae. It has been proposed that Me5 neurons may be involved in relaying kinetic information to the CNS.

The present study was performed to better clarify the functional role of the Me5 sensory innervation of the whisker pad structures by analysing the electrophysiological responses of Me5 neurons to mechanical deflection of the macrovibrissae.

The spontaneous electrical activity of the Me5 neurons, identified by their responses to the masseter muscle stretch, was extracellularly recorded using tungsten-in-glass microelectrodes (impedance 700-900 KΩ) carefully advanced into the Me5 by an electronic microdrive (David Kopf). The electrical signals were relayed to conventional preamplifiers connected to oscilloscopes and then to computers for the specific analysis (Tecfen computerscope analysis ISC-16 software, and PowerLab 4/30 Chart 5, V 5.4.2 software). The spontaneous electrical activity of the Me5 neurons multiunit activity (MUA) was continuously monitored and recorded under resting conditions (*i.e.* vibrissae motionless), during the masseter muscle stretch, and during/after the mechanical deflection of the vibrissae bundle, which was performed in four principal directions (forward/backward, backward/forward, up/down and down/up) using a delicate glass rod connected to a craft-made electronic drive.

The electrophysiological results demonstrated that mechanical deflection of the macrovibrissae induced a significant increase in the spontaneous electrical activity of the Me5 neurons as well as significant changes in the spontaneous firing pattern. It appeared that Me5 neurons could be activated in response to macrovibrissae deflection in specific directions.

The results of the present and previous experiments, allow us to conclude that in addition to the neurons connected to the masticatory muscles[2] and those connected to the periodontal ligament,[3] the Me5 enclose primary neurons specifically dedicated to encoding kinetic information related to vibrissae movements.

It is known that the central terminals of Me5 neurons join the brain

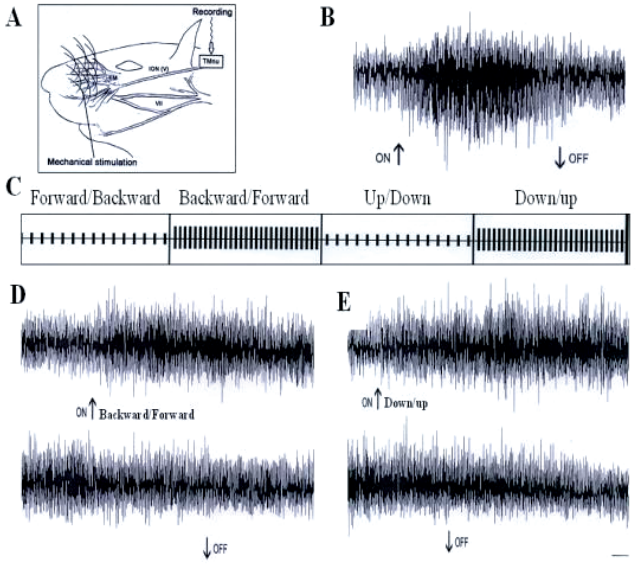

Figure 1. Multiunit electrical activity (MUA) of trigeminal mesencephalic neurons (Me5) recorded in the medial-caudal part of the Me5 during mechanical deflection of the macrovibrissae. A) schematic drawings of the experimental set up; B) specimens showing the Me5 neurons response to mechanical stretch of the masseter muscle to functionally identify the Me5; C) diagrams resuming the four principal directions used during mechanical deflection of the vibrissae bundle together with the schematic pattern of the correspondent Me5 MUA response; D) tonic increase of Me5 MUA, although with adaptation, to vibrissae deflection in back/forward direction; E) tonic increase of the same Me5 MUA to vibrissae deflection in down/up direction, the increased activity lasted throughout the stimulus application as well as several seconds after its end. ON and OFF indicate the beginning and the end of vibrissae displacement. Horizontal calibration: 1 sec; vertical calibration: 1 mV.

Correspondence: Antonella Russo, Physiology Section, Department of Biomedical Sciences, University of Catania, viale A. Doria 6, 95125 Catania, Italy.
E-mail: antrusso@unict.it

Journal of Biological Research 2015; 88:5161

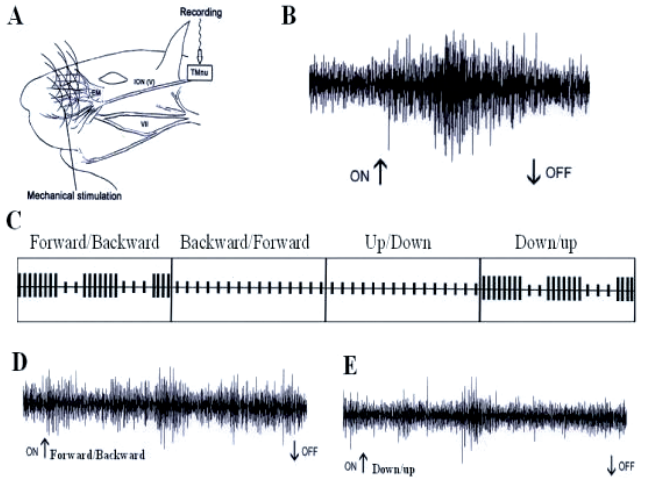

Figure 2. Multiunit electrical activity (MUA) of trigeminal mesencephalic neurons (Me5) recorded in the medial-caudal part of the Me5 during mechanical deflection of the macrovibrissae. A) schematic drawings of the experimental set up; B) specimens showing the Me5 neurons response to mechanical stretch of the masseter muscle to functionally identify the Me5; C) diagrams resuming the four principal directions used during mechanical deflection of the vibrissae bundle together with the schematic pattern of the correspondent Me5 MUA response; D) bursts of Me5 MUA, in response to vibrissae deflection in forward/backward direction; E) the same Me5 MUA responded with bursts, but at longer latency and rapid adaptation, to vibrissae deflection in down/up direction. ON and OFF indicate the beginning and the end of vibrissae displacement. Horizontal calibration: 1 sec; vertical calibration: 1 mV.

stem trigeminal sensory nuclei, which in turn receive, by the primary Gasser's ganglia neurons, tactile information from macrovibrissae receptors. If so, it is possible to hypothesize that the brain stem trigeminal nuclei may deduce higher-order information by combining touch information from macrovibrissae receptors, and carried out by afferents from the Gasser's neurons, with kinetic information arising from the Me5 neurons. The integrated information can be then relayed to more rostral SNC structures to allow a central reconstruction and representation of the spatial displacement of the macrovibrissae.

References

1 Mameli O, Stanzani S, Mulliri G, et al. Role of the trigeminal mesencephalic nucleus in rat whisker pad proprioception. Behav Brain Functions 2010;6:69-79.
2. Darian-Smith I. The trigeminal system. In: Iggo A. (ed.), Handbook of sensory physiology (vol. II). Somatosensory system. Berlin: Springer Verlag; 1973. pp. 271-314.
3. Linden RWA, and Scott BJJ. Distribution of mesencephalic nucleus and trigeminal ganglion mechanoreceptors in the periodontal ligament of the cat. J Physiol 1989;410:35-44.

Could a wrong consumption of cereals influence preteens obesity in Sicily?

G. Russo,[1] D. Di Majo,[2] M. Giammanco[2]

[1]Research Constortium Gian Pietro Ballatore; [2]Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo

Cereals are an important source of carbohydrates in the human contributing to a certain extent also to the need of protein. They constitute a rich source of both nonstarch polysaccharides (dietary fiber) and starch, which together comprise 70-77% of kernel.

The Guidelines for a Healthy Diet in Italy[1] define the opportunity to take, on a daily basis, at least 60% of energy from carbohydrates, and particularly 45% from complex carbohydrates. In Italy, especially in Sicily, these nutrients are contained in traditional food as pasta and bread made starting from durum wheat as grow material. Recently, especially in the bigger towns, there is also widespread consumption of bread made with soft wheat.[2,3]

In the last decade, consumer behavior is changing in Italy[4] and it is clear a trend of decline in consumption of pasta and bread, instead there is an opposite trend for the consumption of products considered as a substitute for bread (brioches, crackers, biscuits, and other baked goods). It is evident how the nutritional profile of the latter products deviates from the bread due to the fat content, that ranges from 5 to 20%. It is also known as the derivatives of durum wheat, preferably whole grain products, are also characterized by a lower glycemic index than products of soft wheat.

So cereals are a key component to set a proper diet. Some studies also report that influence of a

regular and correct consumption of cereals may contribute to reach and to maintain *normal weight*.[5]

In 2011 was carried out investigations with the aim to understand better the relationship and the presence of cereals in the diet of preteen. Moreover, it is known as in Sicily, as well as other areas of southern Europe, there is a serious problem of childhood obesity,[6,7] mainly related to lifestyles considered incorrect and a lower level of education of the population than other European areas that do not registers this phenomenon.

The investigation involved a sample of 1335 subjects, aged between 10 and 13 years, identified through the involvement of 62 schools distributed throughout the region. The sample involved in the investigation was extrapolated according to a stratification that has taken into account the size of the population under study, age ,sex, and distribution by province. For each individual was recorded during the first half of December 2011, thanks to personnel appropriately trained to collect information in a standardized way, a 24-h recall questionnaire (noting the foods eaten in the last 24 hours from the individual). In addition to the parents were asked to complete a questionnaire on the frequency to record information on lifestyle, frequency of intake of cereals. For each individual was recorded weight (kg), height (cm) and body mass index (BMI, Body Mass Index), according to the standards proposed by the International Obesity Task Force.[8] About frequency of the conditions of the weight of the individuals in the sample, the results have confirmed what was already mentioned by other authors for Sicily.[6,7]

Results of the study shows as the population sample, in the various daily meals, have not a correct relationship with cereals, considering the models known for the Mediterranean population. In particular, it is clear the high frequency of subjects who have not took any kind of cereals at breakfast (n=699, 52%) or at morning-snack (n=396, 30%). Moreover 17% of individuals (n=228) has neither taken any kind of breakfast cereal nor at morning-snack; these individuals have access to the primary source of complex carbohydrates, needed daily, only with lunch.

The individuals who regularly eat pasta are characterized by a lower Body Mass Index than those who do not consume habitually; the same is for those who regularly take breakfast, according as noted by other authors in specific studies conducted in the Mediterranean basin,[9] and for individuals who have eaten at least one type of cereal for breakfast.

Regarding this survey it can be stated that further studies are needed to define the extent to which an erroneous presence and distribution in diets of cereals can affect overweight and obesity, particularly among younger age groups of the population. It is evident, however, that it is more necessary than ever to support an effective education campaign that fill the gaps on nutritional knowledge on cereals; contribute to implement pathways on enhancement of cereals mainly on nutritional point of view, with the aim to promoting correct lifestyles and diet patterns better oriented to limit the risks of occurrence in the population of chronic degenerative diseases related to obesity.

Correspondence: Giuseppe Russo, Research Constortium Gian Pietro Ballatore, via dei Pini 21, 90040 Isola delle Femmine (PA), Italy.
E-mail: giusepperusso@ilgranoduro.it

References

1. INRAN. Linee guida per una sana alimentazione italiana. Rome: INRAN; 2003.
2. Russo G, Messina B. Indagini conoscitive per la valorizzazione dei prodotti da forno nel comprensorio del GAL Terre del Gattopardo (Sicilia). Atti dell'8° Convegno AISTEC-Aci Castello (CT). 2011. 250-254 pp.
3. Costanzo E, Liberto M, Russo G. Schede dei pani siciliani. In: Consorzio Gian Pietro Ballatore per la ricerca su specifici settori della filiera cerealicola, 2001. Atlante del Pane di Sicilia. Palermo: Grafiche Renna; 2001.
4. Casini L, Contini C, Marone E, Romano C, 2013. Food habits. Changes among young Italians in the last 10 years. Appetite 2013;68:21-9.

5. Marquart L, Jacobs DR Jr, Slavin JL. Whole grains and health: an overview. J Am Coll Nutr 2000;19:289-90.
6. Baratta R, Degano C, Leonardi D, et al. High prevalence of overweight and obesity in 11e15-year-old children from Sicily. Nutr Metab Cardio Dis 2006;16:249-55.
7. Spinelli A, Lamberti A, Baglio G, et al. OKkio alla SALUTE: sistema di sorveglianza su alimentazione e attività fisica nei bambini della scuola primaria. Risultati 2008. Roma: Istituto Superiore di Sanità; 2009.
8. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. Brit Med J 2000;320:1-6.
9. Demosthenes BP, Antonogeorgos G, Papadimitriou A, et al. Breakfast cereal is associated with a lower prevalence of obesity among 10-12 year-old children: the PANACEA study. Nutr Metab Cardio Dis 2008;18:606-12.

Amino acid profile of four varieties of durum wheat grown in Sicily

G. Russo,[1] M. Giammanco,[2] D. Di Majo,[2] B. Messina[1]

[1]Research Constortium Gian Pietro Ballatore; [2]Physiology and Pharmacology Unit, Department of Legal, Social and Sports Sciences, University of Palermo

The major use of durum wheat is for pasta products, particularly in the European and North American countries, whereas in other areas (Middle East and North Africa) also it is used as couscous and for various types of bread. The durum wheat is therefore a staple food in the Mediterranean and is one of the commodities that characterize the dietary pattern of the *Mediterranean diet*. During the year 2012 were made in Ciminna (Palermo) in Sicily, four fields of experimental cultivation using four different varieties of durum wheat cultivated in Sicily: Simeto, Iride, Duilio and Saragolla. For each variety were set up two different kinds of cultivation tecniques, in order to verify the influence of the environment on the final results. In particular, the technique *A* employed the same seeding density for the 4 varieties (2.4 quintals of seed per hectare), using the pre-sowing and coverage fertilization with respectively 30.6 and 47.25 units of nitrogen, distributed by the use of diammonium phosphate (18/46) in pre-sowing and ammonium nitrate (27%) in coverage; *technique B* employed a seeding density lower for 4 varieties (variable between 1.8 and 22 quintals of seed per hectare); fertilizing in pre-sowing was carried out with sodium superphosphate (that means zero units of nitrogen) and in coverage with 101.79 units of total nitrogen, distributed through the use of ammonium nitrate (27%) used in two different moments after the crop raised. The results show that the protein content of the different batches analyzed stood between 12.9 and 16.1% DM., higher than the average recorded for the Sicilian crops.[1] Investigations on the amino acid profile showed some differences between the different varieties and also between the different cultivation techniques (Table 1). The most represented amino acid was glutamic acid which is also the one who showed greater variability among the four varieties. Even proline showed a variability in the context of the four varieties evaluated. The other amino acids showed slight or no differences among the four varieties evaluated. The survey results are partially comparable with the data available in the literature for other varieties grown in Italy.[2-4]

Table 1. Average values and standard deviation in amino acids content in four varieties of durum wheat grown with two different cultivation techniques in Sicily.

Amino acid	Agronomic technique A g/100 g of protein	SD	Agronomic technique B g/100 g of protein	SD
Alanine	3,4	0,06	3,3	0,07
Arginine	4,5	0,14	4,4	0,11
Aspartic acid	4,6	0,12	4,6	0,14
Glutamic acid	27,1	0,75	27,4	0,81
Glycine	3,6	0,04	3,5	0,04
Histidine	2,3	0,02	2,3	0,03
Isoleucine	3,4	0,05	3,3	0,04
Leucine	6,6	0,08	6,6	0,16
Lysine	2,7	0,08	2,6	0,09
Phenylalanine	4,4	0,13	4,4	0,10
Proline	9,4	0,30	9,5	0,28
Serine	4,5	0,12	4,5	0,05
Threonine	2,7	0,07	2,7	0,07
Thyrosine	2,7	0,07	2,7	0,07
Valine	4,3	0,12	4,2	0,11
Thryptophan (basic hydrolysis)	1,3	0,04	1,2	0,07
Cystine+cysteine	2,1	0,07	2,1	0,09
Methionine	1,7	0,17	1,8	0,04

For the determination of tryptophan was used the method EN ISO 13904:2005 (hydrolysis in a basic environment). For all other amino acids was used the method ISO 13903:2005. The values are reported in g/100 g of protein.

References

1. Russo G, Messina B, Campisi D, et al. Monitoraggio della qualità del grano duro prodotto in Sicilia: risultati delle campagne di raccolto 2007 e 2008. Osservatorio della Filiera Cerealicola Siciliana. 2009. pp. 119-35.
2. Abdel-Hamid Youssef Abdel-Rahman. Amino acid composition of two Italian durum wheat varieties and their semolina. Food Chem 1983;10:155-8.
3. Jiang XL, Tian JC, Hao Z, Zhang WD. Protein content and amino acid composition in grains of wheat-related species. Agr Sci China 2008;7:272-9.
4. Konzak CF. Genetic control of content, amino acid composition, and processing properties of proteins in wheat. Adv Genet 1977;19:407-582.

Correspondence: Giuseppe Russo, Research Constortium Gian Pietro Ballatore, via dei Pini 21, 90040 Isola delle Femmine (PA), Italy.
E-mail: giusepperusso@ilgranoduro.it

Extracellular vesicles can shuttle molecules among brain cells

P. Saladino,[1] G. Schiera,[1] C.M. Di Liegro,[1] P. Proia,[2] I. Di Liegro[3]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [2]Department of Law, Sports, Society and Sciences, University of Palermo; [3]Department of Experimental Biomedicine and Clinical Neurosciences, University of Palermo, Italy

Both tumor and normal brain cells release membrane vesicles (MVs) into the extracellular space.[1-3] MVs shed by astrocytes and neurons contain FGF2 and VEGF, which could be responsible for inducing endothelial cells to form the blood-brain barrier.[2,3] MVs shed from G26/24 oligodendroglioma cells, when added to primary cultures of rat cortical neurons, inhibit neurite outgrowth, and induce apoptosis in about 75% of the cells.[1] The same amount of shed MVs induce apoptosis in about 40% of cultured astrocytes.[4] The analysis of G26/24 vesicles demonstrated the presence of Fas Ligand and TRAIL, which could cooperate in inducing brain cell death.[1,4] The horizontal transfer of labeled proteins from oligodendroglioma cells to astrocytes in culture was also demonstrated.[4] Since MVs were shown to transfer RNA, and, on the other hand, some RBPs are involved in tumorigenesis, we searched for RBPs in MVs. A preliminary analysis in A375 melanoma cells revealed the presence of at least three RBPs, with apparent MW of about 64, 50 and 36 kDa, respectively. These proteins are able to bind H1° mRNA. In developing rat brain, the amount of histone H1° increases during neuronal differentiation, while the level of the corresponding mRNA decreases, suggesting that H1° gene expression is mainly regulated at the post-transcriptional level.[5] Similar results were obtained with cultured astrocytes, while G2624 maintain high levels of both H1° protein and mRNA. Moreover, oligodendroglioma cells, but not astrocytes, release H1° protein into the culture medium by shedding MVs.[6] These findings suggest that oligodendroglioma cells can escape antiproliferative cues by discharging into the extracellular environment molecules expressed concomitant with differentiation, such as H1° histone.

References

1. D'Agostino S, Salamone M, Di Liegro I, Vittorelli ML. Membrane vesicles shed by oligodendroglioma cells induce neuronal apoptosis. Int J Oncol 2006;29:1075-85.
2. Schiera G, Proia P, Alberti C, et al. Neurons produce FGF2 and VEGF and secrete them at least in part by shedding extracellular vesicles. J Cell Mol Med 2007;11:1384-94.
3. Proia P, Schiera G, Mineo M, et al. Astrocytes shed extracellular vesicles that contain fibroblast growth factor-2 and vascular endothelial growth factor. Int J Mol Med 2008;21:63-7.
4. Lo Cicero A, Schiera G, Proia P, et al. Oligodendroglioma cells shed microvesicles which contain TRAIL as well as molecular chaperones and induce cell death in astrocytes. Int J Oncol 2011;39:1353-7.
5. Castiglia D, Cestelli A, Scaturro M, et al. H1(0) and H3.3B mRNA levels in developing rat brain. Neurochem Res 1994;19:1531-7.
6. Schiera G, Di Liegro CM, Saladino P, et al. Oligodendroglioma cells synthesize the differentiation-specific linker histone H1° and release it into the extracellular environment through shed vesicles. Int J Onc 2013;43:1771-6.

Correspondence: Patrizia Saladino, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze 16, 90128 Palermo, Italy.
E-mail: patrizia.saladino@unipa.it

Valutazione della termoporazione dinamica irreversibile come strumento per l'abbattimento della carica batterica in matrici alimentari

L. Saporito,[1] F. Bonura,[1] D. Geraci,[1] T. Rubino,[2] E. Pipitone,[2] M. Cammalleri,[2] G.M. Giammanco,[1] C. Mammina[1]

[1]Department of Sciences for Health Promotion, University of Palermo; [2]Department of Chemical, Management, Informatic, Mechanical Engineering, University of Palermo, Italy

Introduzione

La termoporazione dinamica irreversibile (DIT) consiste in un nuovo tipo di tecnologia di pastorizzazione ideato da Koulik e colleghi[1] basato su un processo termico dinamico, caratterizzato da riscaldamenti molto rapidi (shock termici) a temperature finali non superiori a 70°C. L'obiettivo del nostro studio è stato la valutazione dell'influenza di parametri di processo quali la temperatura di partenza (T_1) del campione da trattare, la temperatura finale in corso di trattamento (T_2), la differenza tra temperatura iniziale e temperatura finale (ΔT), la velocità di riscaldamento (θ) e la durata del trattamento sull'abbattimento della carica batterica nella matrice alimentare.

Materiali e Metodi

I test sono stati effettuati su ceppi di riferimento di microrganismi di possibile interesse alimentare: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae*, *Listeria innocua*. La sospensione test è stata preparata poco prima dell'esecuzione dei test con le seguenti composizioni: 10% sospensione microbica (108 CFU/mL) in acqua peptonata, da una coltura fresca (18-24 ore); 10% sostanza interferente (albumina bovina 0,3 g/L o saccarosio 10 g/L); 80% acqua dura (calcio e magnesio).[2] I test DIT sono stati effettuati fissando per i parametri velocità di riscaldamento, temperatura iniziale e temperatura finale i seguenti valori: DIT 1. θ 20°C/s ; T_1 30°C; T_2 60°C (ΔT 30°C); DIT 2. θ 20°C/s; T_1 30°C; T_2 65°C (ΔT 35°C); DIT 3. θ 30°C/s; T_1 30°C; T_2 60°C (ΔT 30°C); DIT 4. θ 30°C/s; T_1 30°C; T_2 65°C (ΔT 35°C). È stato anche saggiato l'effetto di un prolungato mantenimento della temperatura T_2. A fine trattamento, aliquote di ciascun campione sono state seminate in piastre di terreno solido. La lettura è stata effettuata dopo 24 e 48 ore di incubazione a 37°C. L'attività battericida è stata definita come la dimostrazione di una riduzione di almeno 5 log (logR>5) rispetto alla carica batterica iniziale.

Correspondence: Laura Saporito, Department of Sciences for Health Promotion, University of Palermo, via del Vespro 129, 90127 Palermo, Italy.
E-mail: laura.sapo@tin.it

Journal of Biological Research 2015; 88:5161

Risultati

Le Tabelle 1 e 2 riportano l'efficacia dei trattamenti DIT su ciascun microrganismo. I trattamenti con T_2=60°C e ΔT=30°C (DIT1 e DIT3) hanno dimostrato efficacia solo quando seguiti dal mantenimento della temperatura T_2. Questo risultato è stato osservato su *P. aeruginosa* e meno costantemente su *E. coli*. I trattamenti con T_2=65°C e ΔT=35°C (DIT2 e DIT4) sono risultati efficaci su tutti i microrganismi. Su *E. coli, L. innocua, P. aeruginosa e S. aureus* logR>5 è stata ottenuta anche senza mantenimento della temperatura T_2, e indipendentemente dalla sostanza interferente (Tabella 1). Diversamente, per *E. hirae* logR>5 è stata ottenuta solamente sui campioni trattati con DIT2 o DIT4 seguiti da un prolungato mantenimento di T_2 (Tabella 2). L'efficacia dei trattamenti è stata più marcata e costante per la sospensione contenente saccarosio.

Tabella 1. Trattamenti di termoporazione dinamica irreversibile efficaci su 4 ceppi batterici di interesse alimentare. Numero di test efficaci/numero di test effettuati.

Microrganismo testato	DIT1+ mant.		DIT3+ mant.		DIT2		DIT4	
	A	S	A	S	A	S	A	S
E. coli	1/4	1/4	1/4	0/4	4/4	4/4	4/4	4/4
L. innocua	-	-	-	-	4/4	4/4	4/4	4/4
P. aeruginosa	2/4	4/4	4/4	3/3	4/4	4/4	3/4	4/4
S. aureus	0/4	0/4	0/4	0/4	5/5	5/5	5/5	5/5

A, albumina; S, saccarosio.

Tabella 2. Trattamenti di termoporazione dinamica irreversibile efficaci su *E. hirae*. Numero di test efficaci/numero di test effettuati.

Microrganismo testato	DIT2+mant.		DIT+mant.	
	A	S	A	S
E. hirae	0/4	3/4	1/4	4/4

A, albumina; S, saccarosio.

Discussione

Il trattamento DIT è risultato efficace nel ridurre la carica batterica iniziale di almeno 5 log su tutti i ceppi batterici esaminati con almeno uno dei protocolli testati. Tra i parametri di processo valutati, la temperatura T_2 sembra avere influenzato notevolmente l'efficacia del trattamento, unitamente alla differenza tra temperatura iniziale e temperatura finale (ΔT) del processo DIT. Infatti, gli obiettivi DIT con T_2=60°C e ΔT=30°C (DIT1 e DIT3) non si sono dimostrati efficaci su nessuna delle specie batteriche testate; al contrario, gli obiettivi DIT con T_2=65°C e ΔT=35°C (DIT2 e DIT4), hanno determinato un abbattimento della carica batterica > 5 log per tutte le specie, ad eccezione di *E. hirae*, la cui resistenza ai trattamenti termici è nota in letteratura.[3,4] *E. hirae* è risultato sensibile ai trattamenti DIT 2 e DIT4 solo se seguiti dal mantenimento della temperatura T_2 e in presenza di saccarosio. Per questo microrganismo, in particolare, sarebbe opportuno ottimizzare il trattamento DIT 2 e 4 (ΔT=35°C) cercando di ridurre al massimo il tempo di mantenimento della T_2. La velocità di riscaldamento (θ) e le due sostanze interferenti utilizzate nella preparazione della sospensione test non hanno generalmente influenzato la suscettibilità delle specie batteriche ai trattamenti DIT.

Conclusione

In conclusione, questi test preliminari suggeriscono che il processo DIT può essere efficace nell'ottenere l'abbattimento della carica batterica in un substrato liquido. I parametri che influenzano maggiormente l'efficacia del processo sembrano essere quelli puramente termici: ΔT e T_2. Ulteriori sperimentazioni tenderanno ad adattare i parametri di processo alle esigenze della produzione a livello industriale.

Bibliografia

1. Sterilisation of liquids in hermetically closed vessels; PCT Patent No. 2008/114136; China Patent No. 200880014495; United States Patent Application No. 20110123690
2. UNI EN 1276:2009 Quantitative suspension test for the evaluation of bactericidal activity of chimica disinfectants and antiseptics used in food, industrial, domestic and institutional areas.
3. Spinks AT, Dunstan RH, Harrison T, et al. Thermal inactivation of water-borne pathogenic and indicator bacteria at sub-boiling temperatures. Water Res 2006;40:1326-32.
4. Aguirre JS, Pin C, Rodríguez MR, García de Fernando GD. Analysis of the variability in the number of viable bacteria after mild heat treatment of food. Appl Environ Microbiol 2009;75:6992-7.

N-Valproyl-aminoacids as new potential antiepileptic drugs: synthesis, characterization and *in vitro* studies on stability

V. De Caro, A.L. Scaturro, F.M. Sutera, L.I. Giannola

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

Epilepsy, affecting at least 50 million persons worldwide, is one of the most common neurological disorders. Despite the significant advances in understanding epileptogenic mechanisms and in counteracting their pathological consequences, this clinical condition still has to be faced of treating more effectively the symptoms (epileptic seizures) and of preventing their unfavourable evolution. So far, research has been unsuccessful involved in developing effective antiepileptic drugs (AEDs) capable of preventing the development of the pathogenic process, set in motion by different etiological factors, that leads ultimately to chronic epilepsies.[1,2] So, a substantial need remains to develop new AEDs with better safety, less toxicity, and higher efficacy.[3,4] Valproic acid, VPA, is one of the four most widely prescribed AEDs. Besides its wide use in both generalized and partial epilepsies, VPA has also gained widespread use in recent years for the treatment of bipolar disorders, neuropathic pain and for prophylactic treatment of migraine.[5,6] However the use of VPA is limited by two rare but potentially life-threatening side effects, hepatotoxicity, induced from the formation of metabolite(s) with a terminal double bond, specifically 4-ene-VPA,[7] and teratogenicity, associated with the parent compound itself.[8] In a previous work we reported the synthesis of aminoacidic ester derivatives of VPA as resulted of chemical conjugation of VPA with esters of essential neutral aminoacids, with the aim of modifying the physicochemical properties relevant to bioavailability, such as solubility or lipophilicity, improving the efficacy and reducing unwanted side or toxic effects of VPA.[9] We had reported also the synthesis of N-valproyl-L-tryptophan, that has shown adequate physicochemical characteristics to permeate biological membranes and antiepileptic activity at lower concentration than VPA.[10,11] In this paper, we focused our research on synthesis and characterization of new aminoacidic compounds with potential antiepileptic activity: N-Valproyl-L-Leucine (ValLeu), N-Valproyl-L-methionine (ValMet) and N-Valproyl-L-Histidine (ValHist). The conjugation could consent to obtain VPA derivatives, lacking of structural characteristics usually implicated on VPA teratogenicity, and avoiding formation of possible hepatotoxic metabolites. The aminoacidic derivatives of VPA was successfully obtained covalent linking carboxyl group of drug with aminic group of L-aminoacids, by synthesis involving two main steps. The first step, described in our previous work[9] was modified by adding DMAP as further coupling agent together with DCC. The structures of obtained compounds were assigned on the basis of respective analytical data-sets, FT-IR, MS and ^{1}H and ^{13}C-NMR spectral data. Since the drug lipophilicity is an important factor conditioning brain uptake, the apparent partition coefficient (P_{app}) could be used as simple descriptor of ability to cross the BBB: values of log P_{app} within -0.2 to 1.3 have been described as optimal for cerebral transport; on the other hand higher values than these could reduce the rate of transport inside the membrane.[12,13] Apparent partition coefficient (*Papp*) of ValLeu, ValMet and ValHist were determined in *n*-octanol/phosphate buffer pH 7.4 solution and expressed as Log *Papp*. The determined Log P*app* resulted -0.11,- 1,02 and -1,61 respectively. The Log $D^{pH7.4}$ values indicate that ValLeu, ValMet are adequate to cross biological membranes and in particular BBB barrier while ValHist value is too low, probably due to the fact that was obtained as hydrochloride.

Compared to others drug administration routes, the oral one remains the most preferred as it implies ease of administration as well as high patient *compliance*. However, the transit through the gastrointestinal tract could constitute a limiting step to bioavailability as a consequence of degradation correlated to the environmental pH. In view of a possible administration of ValLeu, ValMet and ValHist by oral route, studies on their chemical stability were performed in simulated gastro-intestinal buffer (37°C, pH 1.2 to 8.0) and monitored by HPLC analysis. The experiments demonstrated that ValLeu, ValMet and ValHist remained unchanged up to 24 h, and did not produce degradation products or potential metabolites. This behaviour indicates high stability at pH conditions of gastro-intestinal tract.

Since compounds containing amide functional group could be susceptible of hydrolysis by plasma and/or cerebral enzymes, our experiments were focused on the evaluation of stability of ValLeu, ValMet and ValHist in these biological environments. Otherwise, plasma stability of drug candidates plays an important role in drug discovery and development; it is essential for maintaining acceptable drug concentration and half-life in order to achieve desirable pharmacological effects.[14]

Experimental data highlighted that ValLeu, ValMet and ValHist remained unmodified up to 24 h in plasma environment. In rat brain homogenate ValLeu, ValMet and ValHist did not undergo cleavage after 24 h, indicating that synthetized compounds have also good stability to cerebral enzymes.

Correspondence: Anna Lisa Scaturro, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, via Archirafi 32, 90123, Palermo, Italy.
E-mail: annalisa.scaturro@unipa.it

Journal of Biological Research 2015; 88:5161

References

1. Arroyo S, Brodie MJ, Avanzini G, et al. Is refractory epilepsy preventable? Epilepsia 2002;43:437-44.

2. Walker MC, White HS, Sander JWAS. Disease modification in partial epilepsy. Brain 2002;125:1937-50.
3. Bialer M, Yagen B. Valproic acid: second generation. Neurotherapeutics 2007;4:130-7.
4. Shimshoni JA, Bialer M, Yagen B. Synthesis and anticonvulsant activity of aromatictetramethylcyclopropanecarboxamide derivatives. Bioorg Med Chem 2008;16:6297-305.
5. Lennkh C, Simhandl C. Current aspects of valproate in bipolar disorder. Int Clin Psychopharmacol 2000;15:1-11.
6. Guidotti A, Dong E, Kundakovic M, et al. Characterization of the action of antipsychotic subtypes on valproate-induced chromatin remodelling. Trends Pharmacol Sci 2008;30:55-60.
7. Silva MFB, Aires CC, Luis PB, et al. Valproic acid metabolism and its effects on mitochondrial fatty acid oxidation: a review. J Inherit Metab Dis 2008;31:205-16.
8. Bojic U, Ehlers K, Ellerbeck U, et al. Studies on the teratogen pharmacophore of Valproic acid analogues: Evidence of interactions at a hydrophobic centre. Eur J Pharmacol 1998;354:289-99.
9. Giannola LI, Lamartina L, De Caro V. Synthesis and characterization of aminoacidic pro-drugs of valproic acid. Pharmazie 1998; 53:829-34.
10. De Caro V, Giandalia G, Siragusa MG, et al. N-Valproyl-L-Tryptophan for CNS-targeting: synthesis, characterization and efficacy in vitro studies of a new potential antiepileptic drug. Med Chem 2011;7:9-17.
11. Sardo P, Rizzo V, Friscia S, et al. Inhibitory effects of N-valproyl-L-tryptophan on high potassium, low calcium and low magnesium-induced CA1 hippocampal epileptiform bursting activity in rat brain slices. J Neural Transm 2012;119:1249-59.
12. Trojnar MK, Weirzchowska-Cioch E, Krzyzanowski M, et al. New generation of valproic acid. Pol J Pharmacol 2004;56:283-8.
13. Gimenez F, Fernandez C, Mabondzo A. Transport of HIV protease inhibitors through the bloodbrain barrier and interactions with the efflux proteins, P-glycoprotein and multidrug resistance proteins. J Acquir Immune Defic Syndr 2004;36:649-58.
14. Di L, Kerns EH, HongY, Chen H. Development and application of high throughput plasma stability assay for drug discovery. Int J Pharm 2005;297:110-9.

Antifungal susceptibilities of species of the *Sporothrix Schenckii* complex isolated in Italy

F. Scordino, I. Pernice, C. Lo Passo, R. Galbo, M.A. Medici, G. Criseo, O. Romeo

Department of Biological and Environmental Sciences, University of Messina, Italy

Introduction

Recent molecular studies showed that the dimorphic fungus Sporothrix schenckii is no longer the only species able to cause sporotrichosis, a cutaneous lymphatic or systemic mycosis particularly frequent in certain geographical areas such as Mexico, Brazil, Peru, and India.[1] In fact, *S. schenckii* can now be recognized as a species complex comprising at least six sibling species: *Sporothrix brasiliensis*, *Sporothrix globosa*, *Sporothrix luriei*, *Sporothrix mexicana*, *Sporothrix pallida* (formerly Sporothrix albicans) and *S. schenckii* sensu stricto.[2,3] Like S. schenckii, all these new species have been reported to cause diseases in humans and in other animals[1,4,5] although the extent of their impact on human infections is not yet completely known. However, infections due to *S. schenckii* have also been reported from other parts of the world, including Europe, where sporotrichosis is considered a rare disease.[6] Nevertheless, in recent years, several clinical autochthonous cases have been described in patients and animals that live in European countries, showing that this pathogenic fungus is more widespread than is now believed.[1,6] At present, there are relatively few works that have evaluated the susceptibility of *S. schenckii* sensu lato to antifungal agents and the drugs tested so far have shown, in general, poor activity especially against *S. pallida*, *S. globosa*, and *S. mexicana*. Therefore, in this study we decided to evaluate the activities of a panel of antifungal drugs against all members of the *S. schenckii* complex with particular reference to Italian isolates. To our knowledge this is the first study that evaluates *in vitro* activities of antifungal agents against a number of *Sporothrix* spp. isolates recovered from clinical and environmental samples in Italy.

Correspondence: Orazio Romeo, Department of Biological and Environmental Sciences, University of Messina, viale F. Stagno d'Alcontres 31, 98166 Messina, Italy.
E-mail: oromeo@unime.it

Journal of Biological Research 2015; 88:5161

Materials and Methods

Fourteen *Sporothrix* spp. were examined in this study (Table 1). Seven of them were environmental *S. pallida* isolates that have already been well characterized in our previous study.[6] The *identity of each isolate was* determined by partial amplification and sequencing of the calmodulin-encoding gene according to recent studies.[2,6] Antifungal activity of seven drugs (Table 1) was evaluated by disk diffusion method according to the procedures reported in the National Committee for Clinical Laboratory Standards (NCCLS) document M44-A.

In this study, a total of 14 clinical and environmental *Sporothrix* spp. were examined to evaluate their susceptibility to a panel of antifungal agents. The resulting values of the in vitro susceptibility of *S. schenckii sensu lato* isolates are shown in Table 1.

All fungal species were resistant to fluconazole, flucytosine and metronidazole whereas were susceptible to nystatin. An excellent broad-spectrum antifungal activity of miconazole was observed against all examined strains. Regarding ketoconazole, different degree of susceptibility were observed. In particular this drug was active against *S. schenckii*, *S. brasiliensis* and *S. mexicana* but for *S. pallida*, *S. globosa* and *S. luriei* was not possible to measure the diameter of the zone of inhibition due to the presence of a high number of resistant colonies.

Discussion and Conclusions

The discovery of genetically different species within the *S. schenckii* population has generated considerable interest on different aspects of their biology including epidemiology, virulence and antifungal susceptibilities. Previous studies have clearly shown that the geographic distribution of members of the *S. schenckii* complex as well as their trends in antifungal susceptibilities are variable[1,2,7] and therefore more attention should be paid in the diagnosis and therapeutic treatment of infections caused by these species. Throughout this work, miconazole, and to a lesser extent nystatin, showed a good activity against all *Sporothrix* species tested while all isolates were resistant to fluconazole, flucytosine and metronidazole which is in agreement with other previous studies.[7] The broad *in vitro* resistance to fluconazole in clinical isolates of the *S. schenckii* complex suggests an intrinsic resistance to this drug. This is an interesting topic for further study, because this drug is considered the second-line treatment for sporotrichosis. One important result of this study is the excellent broad-spectrum antifungal activity displayed by miconazole, an synthetic imidazole antifungal agent which has never been tested against all members of the *S. schenckii* complex so far. Thus, based on our *in vitro* data, we believe that this drug may represent a very promising antifungal agent in the treatment of human and animal sporotrichosis.

Table 1. Fungal strains, species and anitfungal agents used in this study.

Strain	Species	Antifungal agent tested (mean±SD)*:					
		FCN	FY	MZ	MCL	KCA	NY
SPO1	*S. schenckii*	R	R	R	32.50±1.11	29.00±1.22	14.00±2.35
CDM18	*S. schenckii*	R	R	R	22.00±1.35	25.00±1.05	07.00±1.51
SS40	*S. schenckii*	R	R	R	47.00±1,82	49.00±1.87	16.00±1.65
SPA1	*S. pallida*	R	R	R	21.00±1,24	ND	07.00±0.73
SPA2	*S. pallida*	R	R	R	22.00±1.08	ND	09.00±1.04
SPA8	*S. pallida*	R	R	R	19.00±0.86	ND	13.00±1.91
SAM1	*S. pallida*	R	R	R	27.00±1.46	ND	10.00±0.86
BG	*S. pallida*	R	R	R	23.00±1.71	ND	08.00±1.81
BG2	*S. pallida*	R	R	R	21.00±1.38	ND	07.00±1.52
BG6	*S. pallida*	R	R	R	23.00±1.02	ND	11.00±1.31
SS52	*S. brasiliensis*	R	R	R	21.00±1.29	28.70±1.21	09.00±0.50
SS49	*S. globosa*	R	R	R	23.00±1.61	ND	09.00±1.90
FMR9108	*S. mexicana*	R	R	R	22.00±1.10	25.75±2.01	14.00±1.11
KMU2787	*S. luriei*	R	R	R	25.00±1.71	ND	16.00±1.13

*Mean values±standard deviation (SD). FCN, fluconazole; FY, flucytosine; ME, metronidazole; MCL, miconazole; KCA, ketoconazole; NY, nystatin; R, restistant (no alone present); ND, not determined.

References

1. Barros MB, de Almeida Paes R, Schubach AO. Sporothrix schenckii and Sporotrichosis. Clin. Microbiol Rev 2011;24:633-54.
2. Marimon R, Cano J, Gené J, et al. Sporothrix brasiliensis, S. globosa, and S. mexicana, three new Sporothrix species of clinical interest. J Clin Microbiol 2007;45:3198-206.
3. Marimon R, Gene J, Cano J, Guarro J. Sporothrix luriei: a rare fungus from clinical origin. Med Mycol 2008;46:621-5.
4. Morrison AS, Lockhart SR, Bromley JG, et al. An environmental Sporothrix as a cause of corneal ulcer. Med Mycol Case Reports 2013;2:88-90.
5. Rodrigues AM, de Hoog S, de Camargo ZP. Emergence of pathogenicity in the Sporothrix schenckii complex. Med Mycol 2013;51:405-12.
6. Romeo O, Scordino F, Criseo G. New insight into molecular phylogeny and epidemiology of Sporothrix schenckii species complex based on calmodulin-encoding gene analysis of Italian isolates. Mycopathologia. 2011;172:179-86.
7. Marimon R, Serena C, Gené J, et al. In vitro antifungal susceptibilities of five species of Sporothrix. Antimicrob Agents Chemother. 2008;52:732-4.

Possibile ruolo di Sch1 nel *crosstalk* tra i due maggiori *proaging pathways*

G. Taormina, A. Gallo, M.G. Mirisola

Department of Medical and Forensic Biotechnologies, University of Palermo, Italy

Il lievito *Saccharomyces cerevisiae* è ampiamente utilizzato per la comprensione di molti meccanismi cellulari di base ed il suo impiego, come organismo modello nello studio dei processi che controllano l'invecchiamento, si è rivelato uno strumento di grande validità per l'identificazione delle due più importanti vie che modulano l'invecchiamento negli eucarioti. La prima è la via Ras-dipendente, che vede l'attivazione della proteina chinasi A e la conseguente inibizione dei fattori Msn2 e Msn4 che regolano la trascrizione di alcune heat shock proteins, della catalasi citoplasmatica e delle due superossidodismutasi (Sods). La delezione di RAS è infatti in grado di aumentare la sopravvivenza e tale fenotipo viene revertito dalla successiva delezione dei geni che codificano per Msn2, Msn4 e Sod2, mentre alleli attenuati dell'adenilato ciclasi, effettore positivo della chinasi A, aumentano la longevità delle cellule di lievito confermando il ruolo della chinasi A nell'invecchiamento e nella resistenza agli stress.[1-3] Nella seconda via *pro-aging* gioca un ruolo chiave la serina treonina chinasi Sch9, ortologa di Akt e S6K dei mammiferi, e attivata principalmente tramite TOR; ambedue le vie metaboliche convergono sulla proteina chinasi Rim15, quest'ultima quando è attiva impedisce l'espressione di geni coinvolti nella sopravvivenza e nella risposta agli stress.[4,5] È molto interessante il fatto che i mediatori che fanno parte di queste vie cellulari *pro-aging* trovino i loro ortologhi funzionali o strutturali negli eucarioti superiori, ed è stato confermato che i meccanismi fin ora descritti sono sostanzialmente conservati dal lievito fino ai mammiferi.[6] La via di Ras e quella di Sch9 hanno molti elementi in comune, ad esempio stimolano la crescita e la glicolisi e rispondono entrambe ai nutrienti. Sch9 è stato peraltro isolato come soppressore multi copia di un allele termosensibile di Cdc25 il fattore di scambio del nucleotide legato a Ras.[7] Inoltre il carbossiterminale di questa proteina somiglia a una chinasi cAMP-dipendente. Infine, è stato osservato che l'aumento della durata della fase G1 di ceppi di lievito con la delezione di Sch9 può essere compensato da un'iperattivazione della chinasi A. Questi esperimenti suggeriscano una sovrapposizione funzionale delle due vie, tuttavia la contemporanea delezione di Ras2 e Sch9 ha un effetto molto più pronunciato rispetto alle rispettive singole delezioni e i punti di comunicazione o divergenza tra le due vie metaboliche non sono ancora affatto chiari. È in questo contesto che si inserisce il nostro studio. È stato scoperto che l'unità trascrizionale di Sch9 contiene due ORFs, quella più grande che codifica per Sch9, e una a monte all'interno della regione 5' che può codificare per un peptide di 54 aminoacidi di cui non si conosce la funzione e che è stata chiamata Sch1.[8] Nello studio è stato analizzato il ruolo della regione 5'UTR di Sch9 e il suo coinvolgimento potenziale nel processo d'invecchiamento. Abbiamo dimostrato che l'overespressione di Sch1 aumenta la sopravvivenza e la resistenza agli stress nei ceppi deleti su Sch9, e che questo aumento di resistenza è revertito dalla parziale inattivazione della PKA, ciò fa supporre che Sch9 inibisca Sch1 che a sua volta inibisce la chinasi A rappresentando così un link tra la via di Sch9 e quella di Ras fin ora sconosciuto.

Bibliografia

1. Kaeberlein M. Molecular basis of ageing. EMBO 2007;8:907.
2. Fabrizio P, Longo VD. The chronological life span of Saccharomyces cerevisiae. Aging Cell 2003;2:73.
3. Fabrizio P. SOD2 Functions Downstream of Sch9 to Extend Longevity in Yeast. Genetics 2003;163:35.
4. Fabrizio P. Regulation of longevity and stress resistance by Sch9 in yeast. Science 2001;292:288.
5. Wei M. Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9. PLoS Genetics 2008;4:e13.
6. Fontana L. Extending healthy life span from yeast to humans. Science 2010;328:321.
7. Toda T. SCH9, a gene of Saccharomyces cerevisiae that encodes a protein distinct from, but functionally and structurally related to cAMP-dependent protein kinase catalytic subunits. Genes Dev 1988;2:517-27.
8. Di Blasi F. The SCH9 protein kinase mRNA contains a long 5' leader with a small open reading frame. Yeast 1993;9:21-32.

Correspondence: Department of Medical and Forensic Biotechnologies, University of Palermo, via Divisi, 83, 90133 Palermo, Italy.
E-mail: mario.mirisola@unipa.it

Simultaneous saccharification and fermentation of lignocellulosic waste material for *second generation* ethanol production

A. Tropea,[1,2] D. Wilson,[1] R.B. Lo Curto,[2] G. Dugo,[2] P. Saugman,[3] P. Troy-Davies,[4] K.W. Waldron[1]

[1]Institute of Food Research, Norwich, UK; [2]Department of Environmental, Safety, Territory, Food and Health Sciences, University of Messina, Italy; [3]PBI S.A., San José, Costa Rica; [4]The Old Library, Fleetwood, UK

Currently, bioethanol is produced at industrial scale from sugar and starch; however, bioethanol production systems evidence several concerns about competition with food and feed supplies.[1,2] Alternatively, lignocellulosic biomasses such as agricultural wastes, woody biomasses, and lignocellulosic energy crops, today are expected to be the new *second generation* feedstock for bioethanol production because do not compete with food sources.[3,4] Nowadays, industrial bioethanol production is mainly focused on corn, wheat and sugarcane, as well as on highly abundant agricultural wastes. Lignocellulose-containing biomass is mainly composed of hemicellulose (five carbon polymers), cellulose (six carbon polymers) and lignin (phenol polymers) and therefore has to be pre-treated prior of its use in ethanol production by yeast. The use of residual biomass for bioethanol productions has the added advantage of transforming a waste material into a high-value product.[5] The hydrolysis of celluloses and hemicelluloses to hexoses and pentoses is generally achieved by the addition of several different enzymes such as cellulases and hemicellulases.[6] In this study pineapple wastes, a material rich in sugars and lignocellulosic components, were assayed with the purpose of obtaining a valuable product from the residues of the juice and canning industries. Pineapple wastes, enclosing fruit skin and core, were homogenized in a fruit blender. The resulting homogenate, with a dry matter content of 14% (w/w), was diluted with water to a 9% dry matter in a working volume of 1.5 L, and immediately treated at 100°C for 10 min under continuous mixing to inactivate endogenous hydrolytic enzymes and reduce in the same time any microbial spoilage. No further sterilization procedure was adopted. Simultaneous saccharification and fermentation (SSF) was carried out adding together a commercially-available cocktails of cell-wall degrading enzymes and active *Saccharomyces cerevisiae* NCYC 2826 inoculum (approximately 10^7 cells per mL) to the substrate. Fermentation parameters were: 30°C, pH 4.5 and constant stirring at 200 rpm. CO_2 evolution was measured during the fermentation and representative samples of the fermenting substrate were taken at regular intervals. For each sample, ethanol, glycerol, soluble and insoluble sugars were evaluated using GC and HPLC methods. Moreover, total protein determination by Kijeldahal method and Klason lignin were carried out. Substrate initial fibers and soluble sugars were 23.9 and 42.2% respectively (Table 1); at the beginning of the fermentation Glucose and Xylose were the most abundant neutral monosaccharides followed by Galacturonic acid, Arabinose, Galactose and Mannose, with smaller proportions of Rhamnose and Fucose. The main sugars in the soluble fraction were Glucose and Mannose; only small amounts of Galacturonic acid and Galactose was detected. By 21 hours soluble sugars and fiber utilization by the yeast, as well as ethanol production, stopped (Figure 1). The highest ethanol production was 3.7%, reach-

Table 1. Dry matter (DM), fiber and soluble sugars on dry matter, pH, EtOH amount and theoretical yield (TY*), glycerol, protein lignin and ash for SSF process.

	DM%	fiber in DM, %	soluble sugars in DM,%	pH	ethanol (V/V) %		Glycerol %	Protein %	Lignin %	Ash %
					amount	TY %				
Initial	9.0	23.9±2.0	42.2±3.0	4.5	0.1± 0	0.0± 0	0.0± 0	4.1±0.2	3.6±1.0	0.5±0
final	1.7	3.4±0.5	7.5±1.1	3.3	3.7±0.1	96 ± 1	1.0±0.1	17.2±1.5	7.9±0.8	0.6±0

* TY (theoretical yield represents the max ethanol yield: 0.511 g alcohol per 1.0 g glucose.

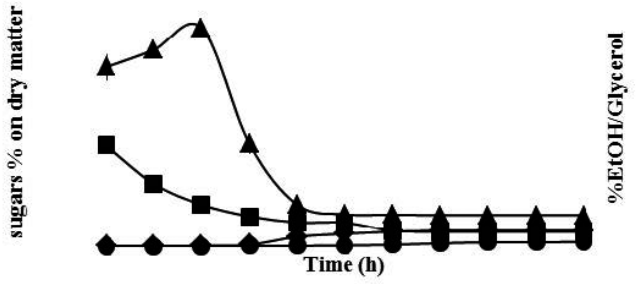

Figure 1. Fiber % (square), soluble sugar % (triangle) calculated on initial dry matter, EtOH % (diamond) and glycerol % (circle) in pineapple waste fermented by *Saccharomyces cerevisiae* NCYC 2826 during simultaneous saccharification and fermentation (SSF).

Correspondence: Alessia Tropea, Department of Environmental, Safety, Territory, Food and Health Sciences, University of Messina, viale F. Stagno d'Alcontres 31, 98166, Messina, Italy.
E-mail: atropea@unime.it

ing a 96% of the theoretical yield (TY). Data about fiber, soluble sugars, ethanol, glycerol, protein, lignin and ash are reported in Table 1. Though the ethanol yield obtained appears rather low, due of course to the low sugar content in the starting material, SSF of pineapple wastes could be attractive since TY, calculated on dry matter loss, reached up 96%, making these wastes an excellent raw material for ethanol production by *S. cerevisiae* NCYC 2826. Moreover substrate resulting from the fermentation process is enriched in protein and lignin, suitable, after separation, for feed and further fuel production respectively.

References

1. Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund MF, et al. Bio-ethanol: the fuel of tomorrow from the residues of today. Trends Biotechnol 2006;24:549-56.
2. Field CB, Campbell JE, Lobell DB. Biomass energy: the scale of the potential resource. Trends Ecol Evol 2008;23:65-72.
3. Galbe M, Zacchi G. Pretreatment of lignocellulosic materials for efficient bioethanol production. Adv Biochem Engin/Biotechnol 2007;108:41-65.
4. Hu G, Heitmann JA, Rojas OJ. Feedstock pretreatment strategies for producing ethanol from wood, bark, and forest residues. BioResources 2008;3:270-94.
5. Goh CS, Tan KT, Lee KT, Bhatia S. Bio-ethanol from lignocellulose: status perspectives and challenges in Malaysia. Biores Tech 2010;101:4834-41.
6. Merino ST, Cherry J. Progress and challenges in enzyme development for biomass utilization. Adv Biochem Engin/Biotechnol 2007;108:95-120.

An HSF2-like factor is present in the invertebrates: characterization and purification in sea urchin embryos and its localization in primary mesenchime cells

G. Turturici, G. Sconzo, F. Geraci

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

The cells respond to environmental, pathological and physiological stresses by inducing the synthesis of the heat shock proteins (HSP) which are highly conserved among all the organisms.[1] The stress response is a common cellular defence mechanism against extracellular stress stimuli. The responsible for the stress-regulated synthesis is the transcription factors (HSF) which activate the transcription of the heat shock genes with a rapid synthesis of their encoded proteins (HSPs). The heat shock proteins are classified into different families on the basis of molecular mass, and one the most conserved during the evolution is HSP70 that is the most abundant and the most reacting HSP to both physiological and environmental stresses. The HSP70 and their cognate proteins (HSCs) function as molecular chaperones to protect cells by binding to partially denatured proteins and dissociating protein aggregates.[2] Single genes for HSF have been cloned from yeast,[3] fruit flies (*Drosophila*),[4] and frogs functionally homologous to mammalian HSF1. Four HSFs have been identified in mammalian and of these, HSF1 and HSF2, are ubiquitously expressed and conserved.[5] HSF1 functions as a classical stress-responsive factor, HSF2 is active during specific development processes and it has been proposed to have a role in developmental processes. Although HSFs are best known as stress- inducible transcriptional regulators, they are also important for physiological processes. HSF functions are from the heat shock response to development, metabolism, disease, especially cancer and neurodegenerative disorders.[6] HSFs contribute to multiple normal physiological processes and pathologies through direct regulation of their target genes. Since reproduction, the immune response and aging are the processes that are affected by the HSF activities an hypothesis would be that these new functions have been recruited during evolution in order to coordinate these processes.[6] In order to verified this hypothesis we investigated whether HSF2-like factor in addition to HSF1 is present in one invertebrate which precedes chordates in evolution. To this aim we demonstrated that in sea urchin *Paracentrotus lividus* embryos are present HSF1 and also HSF2. After characterization and purification we found two HSF2 isoforms located both in the nucleus and in the cytoplasm. α and β sea urchin isoforms seems to be similar to those present in mouse and their expression pattern varies during embryo development, similarly to those of the mammalian HSFs, which are developmentally regulated in a stage-specific manner. In sea urchin the β isoform has greater DNA-binding activity than the α isoform. Moreover, in non-stress conditions the HSE-HSF complex present in early developmental stage embryos is composed predominantly of HSF2, whereas the late developmental stage binding activity is due to HSF1. Studies on territorial localization demonstrate that sea urchin HSF2 is maternal and that during embryo development, until gastrula, is more concentrated in primary mesenchyme cells (PMCs) (Figure 1). Interestingly, Hsp70 distribution shows no spatial correlation with HSF2 expression in non stressed conditions. However, in sea urchin embryos the particular HSF2 localization does not seem to be related to development, because the block of its function, by anti-HSF2 antibody microinjection in eggs, does not disturb the morphogenetic processes after fertilization. It is possible that at its appearance HSF2 did not have any role related to development and this may have been achieved later in evolution.

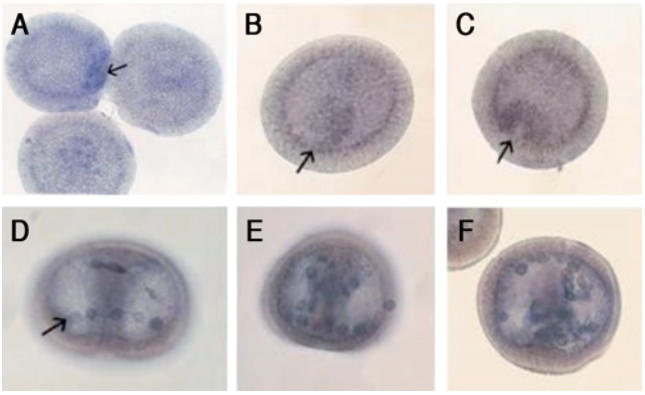

Figure 1. HSF2 localization during embryo development. A-C) Blastula embryos (arrows indicate the ingressing cells); D-F) Gastrula embryos (arrow indicates the primary mesenchyme cell).

Correspondence: Giuseppina Turturici, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze edificio 16, 90128 Palermo, Italy.
E-mail: g.turturici05@libero.it

Journal of Biological Research 2015; 88:5161

References

1. De Maio A. Heat shock proteins: facts, thoughts, and dreams. Shock 1999;11:1-12.

2. Bukau B, Horwich AL. The Hsp70 and Hsp60 chaperone machines. Cell 1998;92:351-66.
3. Wiederrecht G, Seto D, Parker CS. Isolation of the gene encoding the S. cerevisiae heat shock transcription factor. Cell 1988;54:841-53.
4. Clos J, Westwood JT, Becher PB, et al. Molecular cloning and expression of a hexameric Drosophila heat shock factor subject to negative regulation. Cell 1990;63:1085-97.
5. Morano KA, Thiele DJ. Heat shock factor function and regulation in response to cellular stress, growth, and differentiation signals.
6. Akerfelt M, Morimoto RI, Sistonen L. Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol 2010;11:545-55.

Functional characterization of p65(-1), a new isoform of p65 from NF-κB complex

F. Valentino,[1,2] A. Artale,[1] G. Spinelli,[1] F. Gianguzza,[2] F. Di Blasi[1]

[1]Institute of Biomedicine and Molecular Immunology A. Monroy, National Research Council, Palermo; [2]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

Nuclear Factor-κB (NF-κB) are ubiquitous transcription factors that in mammals regulate many biological process including inflammation, immunoregulation, apoptosis, cell growth and cell proliferation.[1] Many studies show that NF-κB is overexpressed in solid and haematological tumours. These data suggest that a deregulated expression of NF-κB pathway is closely related with oncogenic phenotype.[2] NF-κB family members include *RelA* (p65), c-Rel, RelB, p50 and p52. These proteins exert their functions by binding as homodimers or heterodimers to specific DNA target sites (κB consensus). The p65/p50 heterodimer is the most abundant and investigated form of NF-κB[3] A new isoform of p65, named p65(-1), have been discovered in human and mouse. This isoform contains an unknown exon (named exon-1) located upstream to the first known exon of *RelA*, coding for p65. Transcription of the exon-1 leads to an alternative splicing between exon -1 and exon 1, thus skipping exon 0. By consequence p65(-1) has a smaller RHD than p65. Previous evidences show that p65(-1), compared to p65, has different biochemical properties in some cellular mechanism like transcriptional activity on κB consensus, apoptosis and regulation of the glucocorticoid receptor (GR) activation. In this study we investigated the function of p65(-1) by an *in vitro*, *ex vivo* and an *in vivo* approach. In order to test the transcriptional role of p65(-1) we have analysed the transactivation of p65(-1) using both artificial and natural promoter regions, linked with the pathway of NF-κB. We have performed luciferase assays with: NF-κB-Luc (nuclear factor κB) CRE-Luc (cAMP response element), AP1-Luc (AP1 response element), SRE-Luc (serum response element), HSE-Luc (heat shock protein response element), pANXA-1-Luc (annexina 1) e pIL-6-Luc (interleukin 6) to study the activity of p65(-1) and we have also analyzed p65(-1) activity with p65 or p50 under the same conditions. Our data suggest that p65(-1) has a central role during the regulation of pro and anti-inflammatory responses through a specific transcriptional activation using different partners according to the cellular requirements. We have also studied p65(-1) expression on human peripheral blood mononuclear cells (PBMC); it is shown that the expression of mRNA is always present in the analyzed samples. Further, our data demonstrate different expression profiles between individuals considered. The detection of additional factors belonging to NF-κB complex enhances the hypothesis of tuning responses. According with central role of NF-κB in the biological responses we propose to investigate the function of p65(-1) and its network of interaction with other transcriptional factors, in many others cellular processes like apoptosis and cell proliferation.

References

1. Li Q, Verma IM. NF-kappaB regulation in the immune system. Nat Rev Immunol 2002;2:725-34.
2. Kim HJ, Hawke N, Baldwin AS. NF-κB and IKK as therapeutic target in cancer. Cell Death Differ 2006;13:738-47.
3. Verma IM, Stevenson JK, Schwarz EM, et al. REl/NF-κB/IkB family: intimate tales is association and dissociation. Genes Dev 1995;9:2723-35.

Correspondence: Francesca Valentino, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze 4, 90121 Palermo, Italy.
E-mail: francesca.valentino82@gmail.com

Journal of Biological Research 2015; 88:5161

La perossidazione lipidica come esempio di stress ossidativo

S. Vasto,[1] A. Barera,[2] S. Indelicato,[1] D. Nuzzo,[2] M. Di Carlo,[2] C. Caruso[3]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [2]IBIM-CNR, Palermo; [3]Department of Biopathology and Medical Biotechnologies, University of Palermo, Italy

Tra i fattori verosimilmente più implicati nel processo d'invecchiamento (in particolare nell'invecchiamento senza successo, caratterizzato dalla comparsa delle malattie correlate all'età e da disabilità), emerge il ruolo chiave dei radicali liberi, molecole o frammenti molecolari contenenti uno o più elettroni spaiati, e di conseguenza dotati di reattività elevata, e dello stress ossidativo (inteso come condizione ossidante, derivante da uno squilibrio tra fattori ossidanti ed anti-ossidanti a favore dei primi), capaci di indurre infiammazione e danno tessutale.[1] Lo stress ossidativo induce alterazione del bilancio redox delle cellule, con la conseguente attivazione di vie di trasduzione del segnale e di fattori di trascrizioni redox sensibili, capaci di evocare uno stato infiammatorio. Si ha, di conseguenza, un'attivazione continua e prolungata, che determina il rilascio di notevoli quantità di mediatori infiammatori, responsabili della conseguente evocazione di uno stato infiammatorio cronico. L'accumulo con l'età di molecole danneggiate a tutti i livelli (lipidi, proteine e DNA) è indubbiamente uno degli attori principali nei processi d'invecchiamento e di malattie, in quanto può essere responsabile di senescenza cellulare, infiammazione e cancerogenesi.[2] I radicali dell'ossigeno (ROS) e dell'azoto (RNS) appaiono quindi importanti mediatori della risposta infiammatoria da una parte ma responsabili pure del danneggiamento delle cellule dall'altra.[3] Attualmente nei paesi occidentali l'uso di cibi addizionati da integratori antiossidanti sta ricevendo una crescente attenzione e sta per essere sempre più adottato. Un migliore approccio terapeutico e una migliore informazione potrebbe derivare dalla corretta valutazione globale dello stress ossidativo mediante test diagnostici ove le due componenti contrapposte, quella pro- ed anti-ossidante possono essere valutate distintamente.[4] Lo studio si è proposto di valutare in uno studio longitudinale l'effetto dell'assunzione dell'integratore alimentare su 50 soggetti, ugualmente suddivisi per genere in un range di età 50-70 anni. L'integratore contiene tra l'altro polifenoli come; acido Gallico, catechine/epicatechine (monomeri), procianidine dimeri (B1-B4) epolifenoli identificati min. 35%. I biomarcatori valutati nell'ambito dello stress lipidico sono stati gli isoprostani nelle urine e le LDL ossidate nel siero. Ulteriormente, solo nella popolazione maschile è stato valutato il livello di testosterone. In questo studio di coorte prospettico l'integratore alimentare a base di estratti di acido Gallico, catechine/epicatechine (monomeri), procianidine dimeri (B1-B4)ed epolifenoli si è dimostrato capace in esperimenti *ex vivo* di: Influenzare positivamente la concentrazione di LDL ossidate in maniera statisticamente significativa, di avere potenzialità come stimolante nella produzione di testosterone nel genere e di decrementare in maniera statisticamente significativa la concentrazioni di isoprostani nelle urine.

Bibliografia

1. Jacob KD, Noren Hooten N, Trzeciak AR, Evans MK. Markers of oxidant stress that are clinically relevant in aging and age-related disease. Mech Ageing Dev 2013;134:139-57.
2. Mohsenzadegan M, Mirshafiey A. The immunopathogenic role of reactive oxygen species in Alzheimer disease. Iran J Allergy Asthma Immunol 2012;11:203-16.
3. Jenny NS. Inflammation in aging: cause, effect, or both? Discov Med 2012;13:451-60.
4. Gaman L, Stoian I, Atanasiu V. Can ageing be slowed? Hormetic and redox perspectives. J Med Life 2011;4:346-5.

Correspondence: Sonya Vasto, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, via Archirafi, 36 90136 Palermo, Italy.
E-mail: sonya.vasto@unipa.it

Journal of Biological Research 2015; 88:5161

Preparation of solid lipid nanoparticles for ibuprofen delivery

M. Vazzana,[1,2] A.S. Macedo,[2] E.B. Souto,[2,3] C. Faggio[1]

[1]Department of Biological and Environmental Sciences, University of Messina, Italy; [2]Faculty of Health Sciences, Fernando Pessoa University, Porto; [3]Institute of Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

Ibuprofen (IBU), a non steroidal anti-inflammatory drug (NSAID), is largely and currently used for the treatment of several conditions, such as pain, fever and inflammatory reactions. Its therapeutic effects occur through the inhibition of prostaglandin H synthase (PGHS) and cyclooxygenase enzymes (COX-1, COX-2) responsible for the production of prostanoids e.g. prostaglandins.[1] However, IBU exhibits low aqueous solubility, short plasma half-life time of elimination ($t_{1/2}$) and rapid systemic removal, leading to slow dissolution and inadequate tissue absorption with subsequent short duration of its action and poor bioavailability.[2] Thus, current research trends focus on the development of potential delivery systems able to overcome these drawbacks. Notably lipid nanoparticles formulations have been identified as an approach to enhance the rate and extent of drug absorption, as well as, to improve IBU bioavailability.[3] Dynamic colloidal drug carriers based on solid lipid nanoparticles (SLN) may be promising for IBU delivery. SLN consist in a lipid core matrix, which should be solid both at room and body temperatures, with size within 50-1000 nm, stabilized by surfactants in order to prevent size growth during storage.[4] Their main advantages include: small size, ability to penetrate through small capillaries and be taken up by cells allowing the specific drug delivery and targeting, improving the therapeutic effects and reducing toxicity and adverse side effects.[5,6] In addition they show high drug loading capacity, sustained and controlled drug release, long shelf- life, ability to protect the drug from chemical and enzymatic degradation, possibility to be administered by various routes (parenteral, oral, topical, ocular, brain, pulmonary), and they are biocompatible and biodegradable since they are prepared by physiologically well tolerated lipids.[7] The aim of the present work is the one to develop and characterize SLN formulations containing IBU (IBU-SLN). The production process includes a first step based on the lipid screening, performed by using a water bath and temperature above lipid melting point/below drug melting point (≈64°C), in order to select the most suitable lipid accommodating the drug and to evaluate the highest theoretical percentage of drug loading. The next step is based on the choice of adequate surfactants and their concentrations, taking into account their hydrophilic-hydrophobic balance (HLB). After that, the production of the blank formulation is carried on by mean hot high pressure homogenization (HPH) method. In these phases, the choice of the right solid lipid concentration, pressure value and number of cycles are pivotal. The last production step is related to the introduction of the drug in the formulation. The second part of this work is based on the characterization of IBU-SLN through drug entrapment efficiency evaluation, drug release test in sink condition, stability assessment at both room (25°C) and low (4°C) temperatures, pH and conductivity measurements. The entrapment efficiency (EE) is studied using the indirect method with the construction of a previously determined calibration curve by UV-spectrophotometer at 222 nm. By the results of this work, reported in Table 1, what emerges is that Suppocire DM and Witepsol E85 are good lipid candidates for the development of IBU-SLN, stabilized by Phospholipon® 80H and Poloxamer 407 as surfactants. Optical images obtained by microscope (100x magnification) reveal spherical shape particles as shown in Figure 1. pH measurements indicate their poten-

Table 1. Solid lipid nanoparticles composition and their respective pH, conductivity and encapsulation efficiency of the obtained formulations.

SOLID LIPIDS	SURFACTANTS	LOADED DRUG	pH (23°C)	CONDUCTIVITY	EE
Suppocire DM (7%)	Poloxamer 407 (1%) Phospholipon® 80H (0.5%)	1%	4.64	129.04 mV	> 99%
Witepsol E85 (7%)	Poloxamer 407 (1%) Phospholipon® 80H (0.5%)	1%	4.68	124.97 mV	> 93%

Figure 1. Picture of ibuprofen-loaded solid lipid nanoparticles obtained by optical microscope.

Correspondence: Caterina Faggio, Department of Biological and Environmental Sciences, University of Messina, viale Fernando Stagno d'Alcontres 31, 98166 Messina, Italy.
E-mail: cfaggio@unime.it

Journal of Biological Research 2015; 88:5161

tial application for skin; parenteral administration becomes possible only with the increase of pH value *e.g.* by adding NaOH. In fact, it is well-known desirable physical-chemical features depend on the specific considered route of administration. In addition, these formulations show high EE values and they are stable for over 1 month in terms of absence of aggregation and phase separation. However, further investigations are necessary to obtain information about polydispersity index, zeta potential and cytotoxicity in order to allow the application of these formulations for therapeutic purpose in future.

References

1. Rainsford, K.D., Ibuprofen: pharmacology, efficacy and safety. Inflammopharmacology 2009;17:275-342.
2. Dian L. Cubic phase nanoparticles for sustained release of ibuprofen: formulation, characterization, and enhanced bioavailability study. Int J Nanomedicine 2013;8:845-54.
3. Plakkot S. Comminution of ibuprofen to produce nano-particles for rapid dissolution. Int J Pharm 2011;415:307-14.
4. Martins S. Solid lipid nanoparticles as intracellular drug transporters: An investigation of the uptake mechanism and pathway. Int J Pharmac 2012;430:216-27.
5. Goncalves AS, Macedo AS, Souto EB. Therapeutic nanosystems for oncology nanomedicine. Clin Transl Oncol 2012;14:883-90.
6. Souto EB. Feasibility of lipid nanoparticles for ocular delivery of anti-inflammatory drugs. Curr Eye Res 2010;35:537-52.
7. Severino P. Current state-of-art and new trends on lipid nanoparticles (SLN and NLC) for oral drug delivery. J Drug Delivery 2012;2012:1-10.

Gli effetti dell'Acamprosato sulla rimodulazione della trasmissione glutammatergica eccitatoria ed il suo impiego nel trattamento del *craving* da alcolismo nel territorio dell'Azienda Sanitaria Provinciale 1 di Agrigento

F. Venturella,[1] C.M. Bono,[1] S. Aiello,[2] A.M. Ciancio[3]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [2]Department of Law, Society and Sports Sciences, University of Palermo; [3]Provincial Health Unit-Psichiatry Section, Agrigento, Italy

Secondo l'OMS definiamo l'alcolismo quel disturbo a genesi multifattoriale (bio-psico-sociale) associato all'assunzione episodica e/o cronica di bevande alcoliche con presenza o meno di dipendenza capace di determinare una sofferenza multidimensionale che si manifesta in maniera diversa da Soggetto a Soggetto. L'assunzione cronica di alcol modifica la normale attività neuronale attraverso il potenziamento dell'attività inibitoria del GABA e l'inibizione dell'effetto eccitatorio del Glutammato[1] che induce il neuro-adattamento attraverso una over-espressione dei recettori del glutammato in modo da ripristinare l'equilibrio del sistema in presenza di alcol.[2] Quando l'assunzione di alcol viene interrotta l'attività neuronale è caratterizzata sia da un aumento dell'eccitabilità dei recettori del glutammato[1] sia dall'attività dei recettori NMDA che rappresenta invece la causa dei caratteristici sintomi dell'astinenza: le convulsioni.[3] L'Acamprosato è un neuro modulatore specifico per il trattamento della dipendenza da alcol determinando il ripristino dell'equilibrio della trasmissione glutammatergica e l'inibizione dell'attività del glutammato agendo su due recettori:[1] NMDA e mGluR5 rispettivamente ionotropico e metabotropico. Contrastando l'iperattività glutammatergica l'Acamprosato riduce il *craving* negativo e conseguenzialmente diminuisce l'incidenza, la severità e la frequenza delle ricadute.[1] Nello studio clinico effettuato sono stati osservati 30 Pazienti reclutati nel territorio dell'A.S.P.1 di Agrigento suddivisi rispettivamente: >9 Pazienti di cui 2 donne e 7 uomini presso il Ser.T di Sciacca; >6 Pazienti di cui 1 donna e 5 uomini presso il Ser.T di Ribera; >7 Pazienti di cui 2 donne e 5 uomini presso il Ser.T di Agrigento; >8 Pazienti di cui 4 donne e 4 uomini presso il Ser.T di Canicattì. Riportiamo i dati di alcuni Pazienti reclutati e seguiti ambulatorialmente presso i Ser.T che erano già stati sottoposti a precedenti trattamenti farmacologici con GHB: *4 Pazienti sui 30 – pari al 13,33% - esito negativo; *9 Pazienti sui 30 – pari al 30% - esito positivo; *17 Pazienti sui 30 – pari al 56,67% - si sottoponevano per la prima volta alla terapia con Acamprosato. Ciascun Paziente è stato valutato mediante 2 questionari: OCDS (Obsessive Compulsive Drinking Scale) costituito da 14 item e SHORT SLEEP INDEX composto da 4 *item*. Tutti sono stati sottoposti a controlli seriati nel tempo che così abbiamo identificato: – T_0 – prima dell'assunzione di Acamprosato; il primo *follow-up* al 4° mese – T_1 - ed all'8° mese – T_2 - il secondo. Abbiamo, negli esami laboratoristici effettuati, riscontrato i seguenti valori medi (Tabella 1). Per quanto riguarda la valutazione del *craving* si è osservato al T_0 una percentuale dell'86,67% ricovero ed una del 13,33% ambulatoriale. Dove per ricovero si intende la percentuale di Pazienti che, rispondendo alle domande del test, ha totalizzato un punteggio relativo al *craving* >22, valore che richiede un monitoraggio costante da parte del Medico Responsabile del Ser.T e contemporaneamente anche di un maggiore supporto psicologico. Con il termine ambulatoriale si indicano tutti quei Pazienti il cui grado di *craving* risulta al di sotto dei valori considerati a *rischio ricadute* e tali da permettere al Paziente di proseguire un trattamento esclusivamente diurno ma che prevede comunque l'adeguato supporto psicologico all'interno del Ser.T. Al T_1 ed al T_2 si osserva (Figure 1 e 2). Al termine dell'odierno lavoro ed alla luce dei risultati ottenuti è innegabile l'efficacia dell'Acamprosato nel mantenimento dell'astinenza nei Soggetti dipendenti dall'alcol. Efficacia che si è manifestata riducendo il rischio di ricadute da un lato e, dall'altro per i ridotti effetti indesiderati registrati (la diarrea – il prurito) e per la notevole riduzione del *craving* negativo. Possiamo pertanto concludere dicendo che l'Acamprosato, associato ad un opportuno supporto psicologico, può senza dubbio rappresentare la terapia d'elezione che, certamente, in un futuro prossimo, sarà completata da altri supporti farmacologici-psicologici o altro per la riduzione/annientamento del problema: dipendenza dall'alcol.

Correspondence: Fabio Venturella, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze 16, 90128 Palermo, Italy.
E-mail: fabio.venturella@unipa.it

Journal of Biological Research 2015; 88:5161

Tabella 1. Valori medi ematici in diversi periodi del trattamento con Acamprosato.

Valori ematici	T_0 inizio trattamento	T_1 4° mese	T_2 8° mese
Hb	14,56±1 g/dL	14,51±1 g/dL	14,53±1 g/dL
MCV	93,37±1 fl/L	91,3±1fl/L	90,8±1 fl/L
CDT	1,57±1	1,26±1	1,06±1
AST	58,3±UI/L	37,59±1 UI/L	32,93±1 UI/L
ALT	48,73±1UI/L	34,27±1 UI/L	30,07±1UI/L
GGT	209,47±1 UI/L	128,76±1 UI/L	77±1 UI/L

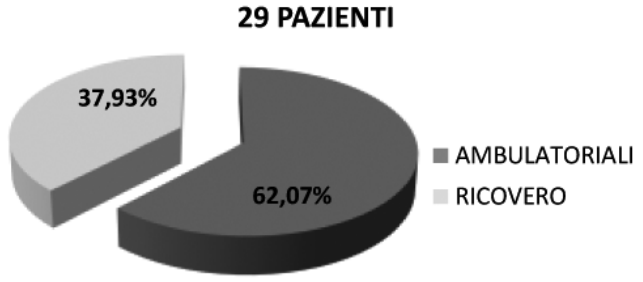

Figura 1. Valutazione *craving* secondo l'Obsessive Compulsive Drinking Scale al quarto mese (T1).

Figura 2. Valutazione *craving* secondo l'Obsessive Compulsive Drinking Scale all'ottavo mese (T2).

Bibliografia

1. De Witte P. Neuroprotective and abstinence-promoting effects of acamprosate. CNS Drugs 2005;19:517-37.
2. Littleton JM. Acamprosate in alcohol Dependence: Implications of a Unique Mechanism of Action. J Addict Med 2007;1:115-25.
3. Mason BJ, Heyser CJ. Acamprosate: a protorypic neuromodulator in the treatment of alcohol dependence. CSN & Neurological Disorders-Drug Targets 2010;9:23-32.

Evaluation of bacterial and fungal load in fresh, frozen and dried food mushrooms

D. Magli,[1] F. Venturella,[2] M.L. Gargano,[1] C. Sciarratta,[3] A. Zummo,[3] G. Venturella[1]

[1]Department of Agricultural and Forest Sciences, University of Palermo; [2]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [3]Provincial Health Unit 6, Palermo, Italy

The analysis carried out by Doores and colleagues[1] and Venturini and colleagues[2] on the total bacterial load tested in wild and cultivated fresh mushrooms highlight values ranging between 3.7 and 9.3 log ufc/g. Due to the absence of pathogens, the microbiological quality of mushrooms analysed by the above-mentioned authors has been considered good. On the basis of data reported in literature we have also extended the observations with frozen and dried mushrooms in order to evaluate the mesophilous bacterial and fungal load. In particular the presence/absence of *Escherichia coli* (Migula,1895) Castellani & Chalmers, 1919, *Salmonella* spp. and *Listeria monocytogenes* (Murray *et al.,* 1926) Pirie 1940 was analysed. The aim of this paper is also to evaluate the quality and safety of mushrooms daily consumption by consumers. Thirty samples of mushrooms (10 cultivated fresh mushrooms identified with letter A 1-10; 10 dried mushrooms identified with letter B 1-10 and, 10 frozen mushrooms identified with letter C 1-10) were taken from large-scale distribution markets, mini-markets and small markets owned by migrants. The frozen mushrooms were contained in packages of *Agaricus bisporus* (J.E. Lange) Imbach 1946 and packages of *Boletus edulis* Bull. Besides mixed packages of *A. bisporus*, *Pholiota nameko* (T. Itô) S. Ito & S. Imai, *Boletus luteus* L., *Pleurotus ostreatus* (Jacq.) P. Kumm. and, *Lentinula edoses* (Berk.) Pegler were analysed. Other analyzed frozen mushrooms were a mixture of *A. bisporus*, *Agrocybe aegerita* (V. Brig.) Singer, *P. ostreatus* and, *P. cornucopiae* (Paulet) Rolland and a mixed package of *A. bisporus* and *P. ostreatus*. The fresh mushrooms (*A. bisporus* and *Pleurotus ostreatus*) were purchaed from the grocery store. Dried mushrooms (*L. edodes*) were purchased from shops owned by Chinese migrants. The microbiological analysis were carried out in the laboratory of the Center of Mycological Control belonging to the Sanitary Agency of the province of Palermo (southern Italy). The total bacterial load was analysed in 25 g of mushrooms (1:10 diluition) according to the rule ISO 4833:2004.[3] Moulds and yeasts were analysed according to the report ISTISAN 96/35.[4] *Salmonella* sp. was checked through the criteria of analysis ruled by UNI EN ISO 6579:2008.[5] The methodology of ISO 16649-2:2001[6] was used to test the *Escherichia coli* positive beta-glucorinidase. *Listeria monocitogenes* was tested accordino to the rule ISO 11290-1:2005.[6] The total count in Petri dishes was made using the formula reported in ISO 7218:2007.[7] The cultura media were provided by the concern Lickson srl (Vicari, province of Palermo). The nomenclature follows the List of Prokaryotic Names with Standing in Nomenclature (LPSN). *L. monocitogenes* and *Salmonella* spp. were not found in the analysed mushrooms. On the contrary a sample of fresh mushrooms from a supermarket of the town of Palermo was polluted by *E. coli*. The count of *E. coli* positive beta-glucorinidase correspond to 1.7 10^4 cfu/g. The value of total bacterial count in all the mushrooms analysed varies from a minimum of 3.8 10^2 cfu/g found in dried mushrooms (*L. edodes*) to a maximum 2.6 10^8 ufc/g in a fresh sample of mixed mushrooms (*A. bisporus* and *P. ostreatus*) in the supermarket. As regards moulds and yeasts the value varies from zero in dried mushrooms (*L. edodes*, *B. edulis*) to 4.4 10^4 ufc/g in fresh mushrooms (*A. bisporus*). The results obtained showed that an analyzed sample of mushroom had a high pollutant load of *E. coli*. As known this bacterium is responsible of intestinal infections that can result in serious extra-intestinal infections. Besides *E. coli* is involved in drug resistance and thus have a significant impact on human health. Since the packaging of fresh mushrooms polluted by *E. coli* was purchased from a supermarket belonging to the mass distribution highlights the need and importance of sanitary controls for the protection of the consumers. The recent warning from the EFSA (European Food Safety Authority) that Italy is the second country in the EU as largest food borne diseases (especially salmonellosis) reinforces the need to respect to meet the parameters set out in Regulation 2073/2005 but also the checking of the proper handling of mushrooms during cultivation and packaging, including compliance with good hygienic practices by insiders.

Correspondence: Giuseppe Venturella, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze 16, 90128 Palermo, Italy.
E-mail: giuseppe.venturella@unipa.it

Acknowledgements: the authors wish to thank Dr. Gaetano Licata (Lickson srl) for his kindly providing cultural media.

References

1. Doores S, Kramer M, Beefman R. Evaluation and bacterial populations associated with fresh mushrooms (Agaricus bisporus). Dev Crop Sci 1986;10:283-94.
2. Venturini ME, Reyes JE, Rivera CS, et al. Microbiological quality and safety of fresh cultivated and wild mushrooms commercialized in Spain. Food Microbiol 2011;28:1492-8.

3. ISO. UNI EN ISO 4833:2004: Metodo orizzontale per la conta di microorganismi. Tecnica della conta delle colonia a 30 gradi. Rome: ISO; 2004.
4. De Medici D, Fenicia L, Orefice L, Stacchini A. Metodi di analisi per il controllo degli alimenti. Rome: Istituto Superiore di Sanità; 1996.
5. ISO. UNI EN ISO 6579:2008. Microbiologia di alimenti e mangimi per animali. Metodo orizzontale per la ricerca di Salmonella spp. Rome: ISO; 2008.
6. Carratù B, Aureli P. Criteri chimici e microbiologici per la valutazione di conformità degli integratori alimentari a base di piante. Rome: Istituto Superiore di Sanità; 2007.
7. ISO. International Standard ISO 7218. Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations. Geneva: International Organization for Standardization; 2007.

Metodi fisico e biologico di identificazione di alimenti irradiati contenenti cellulosa attraverso l'uso della DNA *comet assay* e della spettroscopia di risonanza di spin

M.C. D'Oca,[1] A. Bartolotta,[1] C. Garofalo,[1] A. Parlato,[2] A.M. Di Noto,[3] C. Cardamone[3]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [2]Department of Energy, Information Engineering and Mathematical Models, University of Palermo; [3]The Institute for Experimental Veterinary Medicine of Sicily, Palermo, Italy

Il trattamento a scopo conservativo degli alimenti tramite radiazioni ionizzanti prevede l'uso di dosi non superiori a 10 kGy, in grado di ridurre la flora microbica iniziale, inibire la germogliazione, aumentando così il grado di conservazione e di sicurezza dell'alimento. In Italia tale trattamento è applicabile solo ad aglio, cipolla, patata e spezie, ma si ritiene che possano essere introdotti nel mercato alimenti irradiati, provenienti da paesi in cui possono essere irradiati numerose tipologie di alimenti. Per tale ragione il Dl.vo n. 94 del 30.01.01, prescrive che tutti gli alimenti irradiati immessi sul mercato debbano riportare in etichetta la dicitura *irradiato* e che le autorità sanitarie debbano effettuare controlli sui prodotti in fase di commercializzazione. A tale scopo il Comitato Europeo di Normalizzazione ha emanato dei protocolli per l'utilizzo di metodi di identificazione di alimenti irradiati, distinti in metodi Fisici, Chimici e Biologici, a loro volta distinguibili in metodi di screening e di conferma. Il presente lavoro riporta i risultati relativi alla messa a punto e validazione di un metodo di *Screening* Metodo Biologico di screening UNI EN 13784:2002-DNA *comet assay* e di un metodo di conferma Metodo Fisico di conferma UNI EN 1787:2000-Metodo per spettroscopia di risonanza di spin (ESR). La matrice alimentare su cui sono state applicate ambo due le procedure sono alimenti vegetali contenti cellulosa, che possono essere presenti nel mercato nazionale sia come prodotti autoctoni sia come prodotti importati da paesi comunitari ed extracomunitari, ove possono vigere normative differenti nell'ambito dell'irragiamento alimentare, quindi di forte interesse nell'ambito dei controlli ufficiali da parte di enti autorizzati al controllo. Nello specifico le matrici analizzate sono: pistacchi, paprica e fragole. E le analisi sono state condotte sia su campioni non irradiati, usati come bianco campione, che su campioni irradiati a dosi basse e medie. Sono state effettuate prove di ripetibilità e riproducibilità del metodo sia sui campioni non irradiati che irradiati, che hanno permesso di standardizzare e quindi validare i due metodi di identificazione degli alimenti irradiati. In Figura 1 sono mostrate le immagini relative all'analisi di *screening* DNA *comet assay* applicata ad un campione di pistacchio prima e dopo irraggiamento a 5 kGy; tale tecnica si basa su un'analisi elettroforetica dei campioni. La presenza di una scia, appunto *cometa* nel campione irradiato (1B), è conferma dell'avvenuto trattamento radiante, dovuta alla rottura della membrana cellulare a causa delle radiazioni ionizzanti e quindi migrazione del fluido intracellulare tra i due poli della cella elettroforetica. Contrariamente nell'immagine del campione non irradiato (1B) si evidenzia nettamente la cellula intatta e quindi nessuna cometa. In Figura 2 sono mostrati gli Spettri ESR di un campione di pistacchio prima e dopo irraggiamento a 5 kGy. Tale tecnica permette di rilevare la presenza di molecole paramagnetiche, come i radicali radioindotti. La Figura 2A mostra il tipico segnale ESR di un campione di cellulosa non irradiato, caratterizzato da un singoletto, definito *endogeno*, in quanto non dovuto al trattamento radiante bensì a caratteristiche paramagnetiche della molecola; la Figura 2B invece mostra il tipico segnale della cellulosa irradiata, caratterizzato dalla comparsa di 2 linee satelliti rispetto al segnale endogeno alla distanza di 60 Gauss l'una dall'altra, che risulta essere l'indice dell'avvenuto irraggiamento del campione.

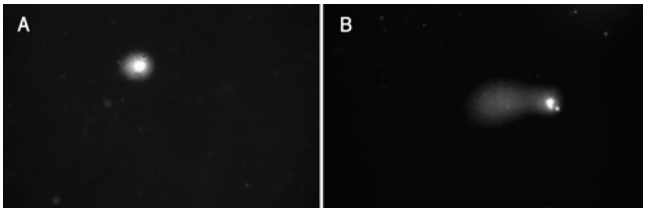

Figura 1. Immagine relativa all'analisi di *screening* DNA *comet assay* applicata ad un campione di pistacchio prima (A) e dopo irraggiamento a 5 kGy (B).

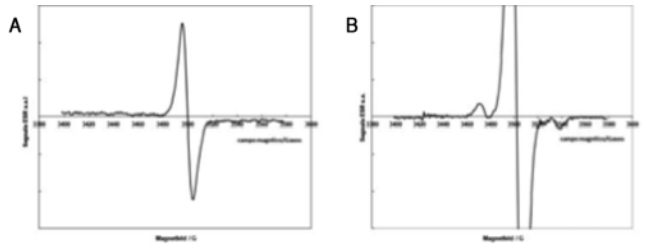

Figura 2. Spettri di risonanza di spin di un campione di pistacchio prima (A) e dopo irraggiamento a 5 kGy (B).

Correspondence: Maria Cristina D'Oca, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, viale delle Scienze 16, 90128 Palermo, Italy.
E-mail: mariacristina.doca@unipa.it

Journal of Biological Research 2015; 88:5161