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Book of Abstracts

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Department for Life Quality Studies, University of Bologna, Italy

Cristina Angeloni
School of Pharmacy, University of Camerino, Italy

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The role of the vascular system in flavonoid-induced human cognitive enhancement

Jeremy P E Spencer

Molecular Nutrition Group, School of Chemistry, Food and Pharmacy, University of Reading, Reading, UK

Evidence suggests that dietary phytochemicals, in particular flavonoids, may exert beneficial effects on the central nervous system by protecting neurons against stress-induced injury, by suppressing neuroinflammation and by improving cognitive function. Historically, they were believed to do this via an ability to express classical antioxidant activity in the brain. However, their poor brain bioavailability and extensive metabolism means that this is unlikely. Instead, their actions on the brain appear to be mediated by effects on both the peripheral and cerebro-vascular system that lead to improved blood flow to the brain capable of inducing enhanced activity within specific domains of cognitive function. Such vascular effects may also lead to the activation of critical protein and lipid kinase signaling cascades in the brain, leading to a suppression of neuroinflammation and the promotion of synaptic plasticity. This paper will focus on the acute and chronic effects of flavonoid and flavonoid-rich food intake on human executive function (attention, sustained attentiveness and task responsiveness) and episodic memory and how such effects may be mediated by changes in peripheral and cerebrovascular blood flow, measured using flow-mediated dilatation and fMRI. Through such a mechanism, the consumption of flavonoid-rich foods throughout life holds the potential to limit neurodegeneration and to prevent or reverse age-dependent loses in cognitive performance. In addition, flavonoids may represent important precursor molecules in the quest to develop a new generation of brain enhancing drugs.

Neuroprotective Potential of Dietary Polyphenols in Dementia

Robert J Williams

Department of Biology and Biochemistry, University of Bath, UK

Epidemiology suggests that dietary polyphenol intake can be inversely related to subjective cognitive decline and to the risk of developing dementia although findings overall are mixed due to diversity in the polyphenols involved and the outcome measures employed. A systematic approach is needed to identify the functional forms of bioavailable polyphenols capable of mitigating risk and their mechanisms of action firmly established. Alzheimer's Disease (AD) is characterised by accumulation of Amyloid β (Aβ) plaques and tau tangles and inhibiting their emergence, particularly during prodromal phases offers potential for slowing disease onset. Polyphenols have been shown to modulate the production, aggregation, post translational modification and clearance of Aβ and tau although there is a lack of mechanistic insight particularly with respect to dietary metabolites. Adopting in vitro polyphenol screening in neurons using an APP-GAL4 assay we showed that (-)-epicatechin (EC) inhibited pro-amyloidogenic APP processing and lowered Aβ. Consistent with this, oral administration of EC reduced Aβ pathology in APP/PS1 transgenic mice. The same oral EC administration regime also inhibited progressive tau pathology in rTg4510 mice independent of actions at APP processing. Based on predicted target engagement we considered that inhibition of GKS3β might explain these phenotypes, but we could not demonstrate inhibition of GSK3β activity with EC either in vivo or in vitro. To gain a better understanding of neuroprotective pathway(s) activation we are undertaking whole RNA sequencing to define polyphenol-response signatures. Future work will focus on bioavailability and metabolism of dietary polyphenols including the influence of the microbiome.

Huntington's disease therapy by small molecules that influence the unfolded protein response

Gerardo Z. Lederkremer

The Shmunis School of Biomedicine and Cancer Research, Cell Biology Division, George Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel
and Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

Protein aggregation and the induction of ER stress stand at the center of many neurodegenerative diseases. Huntington's disease (HD) is caused by a mutation in the huntingtin gene, resulting in expansion of polyglutamine repeats, misfolding and aggregation. We have previously found strong induction of ER stress by toxic mutant huntingtin (mHtt) oligomers, which is reduced upon sequestration of these oligomers into large aggregates. We recently established that the small molecule pridopidine, a sigma-1 receptor agonist, significantly reduces mHtt-induced ER stress in cellular HD models. Pridopidine reduced the levels of markers of the three branches of the unfolded protein response (UPR), showing the strongest effects on the PKR-like endoplasmic reticulum kinase (PERK) branch. This is accomplished by sigma-1 receptor – mediated recruitment of toxic mHtt oligomers into less toxic large SDS-insoluble aggregates, which in turn reduces its induction of ER stress and cytotoxicity.
Our studies and those of others show that the PERK pathway is key to neuronal homeostasis in neurodegenerative diseases. We developed a potent small molecule PERK activator, MK-28. We tested the new compound on cellular HD models and on the R6/2 transgenic HD mouse model. Cells expressing mHtt showed significantly increased survival upon ER stress. MK-28 treated mice showed significant improvement in their motor performance and physiologic measurements and extended lifespan, suggesting that PERK activation postponed the appearance of HD symptoms. Boosting the UPR appears to be a promising new potential therapy for HD and possibly for the treatment of other neurodegenerative diseases.

Addressing the challenges of Alzheimer's disease through multi-target-directed ligands

M.L. Bolognesi

Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, via Belmeloro 6, 40 126 Bologna, Italy, Bologna, Italy

AD affects 7 million people in the European Union (EU), and this figure is expected to double in the next 20 years. It currently costs nearly €130 billion per annum to care for people with AD across EU. As recognized by Horizon EU, which boost health innovation for vulnerable older people, AD is a leading medical and societal challenge.
Despite intensive fundamental and clinical research efforts, there are currently no treatments with clear disease-modifying activity or effective preventive strategies.[1] In June 2021, the U.S. Food and Drug Administration approved the antibody aducanumab, despite ongoing concerns about its safety, efficacy, and cost.[2] All controversy aside, the fact that it is the first new approval since 2003, demonstrates that AD drug development suffers an unsustainably high failure rate.
In the last decade, a new concept based on so called multi-target-directed ligands, i.e., single molecular entities modulating multiple AD targets simultaneously, has been met with a certain success within the drug discovery community. This is because they seem more adequate to face AD complex etiology, rather than conventional single-target drugs. Recent success stories, together with the general notion that natural products have an intrinsic multi-target mechanism of action, have fueled research efforts into the drug development of natural products and natural product-derived compounds as multi-target-directed ligands. In a joint project with the University of Brasilia, we developed new molecules for AD, by exploiting the cashew nut shell liquid.[3] Particularly, we started a search for bioactive templates, which, through a judicious medicinal chemistry-guided structural modification of the natural scaffold, might lead to the discovery of new, effective, multi-target-directed ligands against AD.

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2. *https://www.nytimes.com/2021/06/07/health/aduhelm-fda-alzheimers-drug.html*
3. *Uliassi E. et al, Molecules 2021; 26(18):5441.*

Nutraceuticals for mental illness: Emerging concepts and current state of the evidence

Dr Wolfgang Marx

Food & Mood Centre, Deakin University, Australia

Nutraceutical interventions have received increasing interest from the research, clinical and general community. This increasing interest is partly attributable to the evolving understanding of the neurobiological underpinnings of mental illness, which implicates certain nutrients as potential adjunctive treatments. There is a broad array of nutraceutical interventions that target pathways implicated in mental illness, including inflammation, oxidative stress, mitochondrial dysfunction, and neurotransmitter pathways. Drawing on meta-analytic evidence and recently developed clinical guidelines, this presentation will provide an overview of the efficacy, safety, and clinical considerations regarding the use of nutraceuticals in the management of mental illness. The use of omega-3 fatty acids (particularly as eicosapentaenoic acid) as an adjunctive treatment for depression has the strongest evidence. Other nutraceutical interventions with supportive randomized controlled trial data include folate-based supplements, N-acetylcysteine, as well as phytoceutical preparations such as curcumin. However, there are several nutraceuticals that have only weak or no support for their for potential use; these will also be discussed in this presentation. Furthermore, most research to date has focused on depression, which represents a key gap in the current literature. Finally, this presentation will highlight key areas of emerging research including the role of nutraceuticals that target the gut-brain axis.

Modulating the Neurophysiological Processes Supporting Motor Memory Consolidation to Enhance Performance

Genevieve Albouy[1,2]

[1]*Department of Health and Kinesiology, College of Health, University of Utah, Salt Lake City, UT, USA*
[2]*Department of Movement Sciences, KU Leuven, Leuven, Belgium*

While the neural correlates underlying motor sequence learning and motor memory consolidation are well described, it remains unclear whether these processes can be modulated in order to influence motor performance. Here, I will present a set of recent studies from our group testing whether stress, induced experimentally using the socially evaluated cold pressor test, can modulate the behavioral and neural correlates of motor learning and the subsequent sleep-related motor memory consolidation process. I will present data showing that stress alters brain responses in task-relevant brain areas during the learning process. Importantly, I will describe how inter-subject variability in brain responses to stress determines the impact of stress on motor behavior during learning and after a consolidation interval.

Physical activity, exercise and muscle-brain crosstalk

Bente Klarlund Pedersen*, MD DMSc, Professor

Director of Centre of Inflammation and Metabolism (CIM) and Centre for Physical Activity Research (CFAS) Rigshospitalet and University of Copenhagen, Denmark

Skeletal muscle secretes several hundred myokines that facilitate communication from muscle to other organs, such as, adipose tissue, pancreas, liver, gut, and brain. The biological roles of myokines include effects on e.g glucose and lipid metabolism, tumour metabolism, inflammation as well as brain function.
Exercise has many beneficial effects on brain health, contributing to decreased risks of dementia, depression and stress, and it has a role in restoring and maintaining cognitive function and metabolic control. The fact that exercise is sensed by the brain suggests that muscle-induced peripheral factors enable direct crosstalk between muscle and brain function. Muscle secretes myokines that contribute to the regulation of hippocampal function. Evidence is accumulating that the myokine cathepsin B passes through the blood-brain barrier to enhance brain-derived neurotrophic factor production and hence neurogenesis, memory and learning. Exercise increases neuronal gene expression of FNDC5 (which encodes the PGC1α-dependent myokine FNDC5), which can likewise contribute to increased brain-derived neurotrophic factor levels. Serum levels of the prototype myokine, IL-6, increase with exercise and might contribute to the suppression of central mechanisms of feeding. Exercise also increases the PGC1α-dependent muscular expression of kynurenine aminotransferase enzymes, which induces a beneficial shift in the balance between the neurotoxic kynurenine and the neuroprotective kynurenic acid, thereby reducing depression-like symptoms. Myokine signaling, other muscular factors and exercise-induced hepatokines and adipokines are implicated in mediating the exercise-induced beneficial impact on neurogenesis, cognitive function, appetite and metabolism, thus supporting the existence of a muscle-brain endocrine loop.

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Physical activity and Alzheimer's Disease: is it a helpful co-adjuvant treatment?

Alejandro Santos-Lozano, PhD

Department of Health Sciences, European University Miguel de Cervantes (Spain)

Alzheimer's disease (AD) is a multifactorial and heterogeneous disorder characterized by the interaction of genetic and epigenetic factors and the dysregulation of several intracellular signaling and cellular/molecular pathways. Physical activity/exercise may play a relevant role in the main pathophysiological processes involved in the risk of developing AD: i) Immune system and inflammation; ii) Endothelial function and cerebrovascular insufficiency; iii) Apoptosis and cell death; iv) Intercellular communication; v) Metabolism, oxidative stress and neurotoxicity; vi) DNA damage and repair; vii) Cytoskeleton and membrane proteins; vii) Synaptic plasticity. Also, exercise interventions appear to exert multi-domain benefits in patients with AD, including cognitive function (mini-mental state examination test), physical function (six-minute walking test [6MWT]), functional independence (Barthel index), and neuropsychiatric symptoms (Neuropsychiatric Inventory [NPI]).

DIETARY SUPPLEMENTS, NUTRACEUTICALS, AND FUNCTIONAL FOODS IN NEUROPROTECTION

Neuroprotective activity of novel cannabis phytochemicals: the next horizons in medicinal and nutraceutical cannabis use

Scott Smid, John Staton Laws, Carly Eggers & Dylan Marsh

The University of Adelaide (Australia)

Dementia and other neurodegenerative conditions carry an enormous and ever-increasing health burden to an ageing society. Cannabis is replete with hundreds of unique and understudied phytocannabinoids, in addition to other novel phytochemicals that may possess a 'pot-pourri' of uncharacterized bioactivities. Botanical extracts of medicinal cannabis are an emergent therapeutic class globally, yet little is known regarding the instrinsic bioactivities of these myriad components.

We have investigated several novel cannabis phytochemical candidates for neuroprotective bioactivity in a variety of neuronal cell lines to model protection against insults integral to the neurodegenerative condition Alzheimer's Disease (AD). Neuronal PC-12 and NCS-34 cell lines were exposed to oxidative stress and the neurotoxic misfolding protein amyloid $\beta_{1\text{-}42}$ (Aβ42), alone or in the presence of select cannabis phytochemicals. Candidates included THC, cannabidiol, cannabichromene, cannabidivarin, cannabigerol and cannabinol, several cannabis terpenoids and the novel cannabis flavonoid, cannflavin A.

Cannabichromene, cannabigerol and cannabinol in addition to the terpenes α-bisabolol and β-caryophyllene all inhibited neurotoxicity in response to Aβ42 exposure. Cannflavin A was the most efficacious neuroprotective agent, providing significant protection to PC-12 cells against all Aβ42 exposure concentrations. However neither the phytocannabinoids, cannflavin A or terpenes provided significant protection against lipid peroxidation arising from *tert*-butyl hydroperoxide exposure, while cannabichromene and cannabinol protected neuronal morphology and directly inhibited Aβ42 aggregation.

These studies reveal a profile of neuroprotective activity of novel non-psychotropic cannabis phytochemicals against amyloid β neurotoxicity ; in particular, novel minor phytocannabinoids, select terpenes and prenylated flavones such as cannflavin A that may guide not only the further development of new therapeutic areas for medicinal cannabis use but also nutraceutical formulations preventative of age-related cognitive decline and dementia.

Treatment with oleic acid, linolenic acid and curcumin exerts positive effects on neurodevelopmental alterations in a model of Down syndrome

Renata Bartesaghi[1], Noemí Rueda[2], Susana García Cerro[2], Verónica Vidal[2], Alba Puente[2], Carmen Martínez-Cué[2]

[1] *Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Bologna, Italy*
[2] *Department of Physiology and Pharmacology, Faculty of Medicine, University of Cantabria, Santander, Spain*

Down syndrome (trisomy 21), a pathology caused by triplication of chromosome 21, is characterized by intellectual disability (ID) from the earliest life stages. ID is largely attributable to impaired proliferation of neural progenitor cells (NPCs) in the prenatal period and impaired neuron maturation in terms of dendritic development and connectivity. Increasing efforts are currently underway aimed at establishing whether it is possible to protect pharmacologically neural progenitor cells from the damage to proliferation and differentiation caused by gene triplication. To this purpose various drugs have been tested in the Ts65Dn mouse, a widely used model of DS. Some of these drugs have proven effective but their translational value may be limited by caveats in their use, especially during pregnancy, the more critical period of brain development. In order to establish whether it is possible to protect the trisomic brain from the neurodevelopmental alterations caused by gene triplication via nutraceuticals, we have tested the effects of oleic acid, linolenic acid and curcumin in the Ts65Dn model. Mice were treated with each of these substances during either fetal or neonatal life stages. We found that all tested therapies exerted some benefit on neural precursor proliferation, neuron maturation and cognitive performance. These results may pave the way to the design of clinical trials during gestations, in order to counteract the adverse effects of DS on brain development

Hericium erinaceus and *Coriolus versicolor* Modulate Molecular and Biochemical Changes after Traumatic Brain Injury

Daniela Impellizzeri[1], Rosalba Siracusa[1], Marika Cordaro[2], Roberta Fusco[1], , Ramona D'Amico[1], Salvatore Cuzzocrea[1], Vittorio Calabrese[3], Rosanna Di Paola[1]

1 *Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University of Messina, 98166 Messina, Italy*
2 *Department of Biomedical, Dental and Morphological and Functional Imaging, University of Messina, 98125 Messina, Italy*
3 *Department of Biomedical and Biotechnological Sciences, University of Catania, 95124 Catania, Italy*

Traumatic brain injury (TBI) is a major health and socioeconomic problem affecting the world. This condition results from the application of external physical force to the brain which leads to transient or permanent structural and functional impairments. TBI has been shown to be a risk factor for neurodegeneration which can lead to Parkinson's disease (PD) for example. In this study, we wanted to explore the development of PD-related pathology in the context of an experimental model of TBI and the potential ability of *Coriolus versicolor* and *Hericium erinaceus* to prevent neurodegenerative processes. Traumatic brain injury was induced in mice by controlled cortical impact. Behavioral tests were performed at various times: the animals were sacrificed 30 days after the impact and the brain was processed for Western blot and immunohistochemical analyzes. After the head injury, a significant decrease in the expression of tyrosine hydroxylase and the dopamine transporter in the substantia nigra was observed, as well as significant behavioral alterations that were instead restored following daily oral treatment with *Hericium erinaceus* and *Coriolus versicolor*. Furthermore, a strong increase in neuroinflammation and oxidative stress emerged in the vehicle groups. Treatment with *Hericium erinaceus* and *Coriolus versicolor* was able to prevent both the neuroinflammatory and oxidative processes typical of PD. This study suggests that PD- related molecular events may be triggered on TBI and that nutritional fungi such as *Hericium erinaceus* and *Coriolus versicolor* may be important in redox stress response mechanisms and neuroprotection, preventing the progression of neurodegenerative diseases such as PD.

Effects of the probiotic formulation SLAB51 in *in vitro* and *in vivo* Parkinson's disease models

Vanessa Castelli[1], Margherita Alfonsetti[1], Michele d'Angelo[1], Annamaria Cimini[1]

1 Department of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy

Parkinson is a common neurodegenerative disorder, characterized by motor and non-motor symptoms, including abnormalities in the gut function, which may appear before the motor sign. To date, there are treatments that can help relieve Parkinson' disease (PD)-associated symptoms, but there is no cure to control the onset and progression of this disorder. Altered components of the gut could represent a key role in gut-brain axis, which is a bidirectional system between the central nervous system and the enteric nervous system. Diet can alter the microbiota composition, affecting gut-brain axis function. Gut microbiome restoration through selected probiotics' administration has been reported. In this study, we investigated the effects of the novel formulation SLAB51 in PD. Our findings indicate that this probiotic formulation can counteract the detrimental effect of 6-OHDA *in vitro* and *in vivo* models of PD. The results suggest that SLAB51 can be a promising candidate for the prevention or as adjuvant treatment for PD.

Microbial-derived metabolites: an unexplored risk factor of age-related cognitive decline and dementia

Emily Connell, Gwenaelle Le Gall, Saber Sami, Michael Müller and David Vauzour

Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom. Emails: E.Connell@uea.ac.uk ; D.Vauzour@uea.ac.uk

Due to our progressively ageing global population, the prevalence of age-related cognitive decline and dementia is increasing worldwide and remains without a cure. Identifying and modifying risk factors associated with cognitive decline can enable early detection, minimise disease progression and improve public health. Novel perspectives highlight a bidirectional communication system between the gut, its microbiome and the central nervous system, known as the microbiota-gut-brain axis. Disturbances in this communication system (i.e., dysbiosis) has been associated with increased cytotoxic metabolite production, promoting neuroinflammation and cognitive decline. Using liquid chromatography-tandem mass spectrometry, we compare specific microbial-derived metabolites previously linked with cognitive impairment (e.g., secondary bile acids, trimethylamine-N-oxide (TMAO), p-cresol and tryptophan derivatives) in human serum samples with various stages of early cognitive decline (50 cognitively healthy subjects, 50 subjective memory impairment and 50 mild cognitive impairment patients, matched for age, sex and BMI). The resulting metabolite panel that significantly correlates with cognitive decline provides novel insight into key mediators of early disease progression before irreversible damage may occur. These modifiable risk factors can offer vital insight into the promotion of healthy ageing and cognition, having important clinical implications in today's growing elderly population.

A novel *C. elegans*-based platform for screening anti-tau compounds

Luisa Diomede[1], Carmina Natale[1], Gloria Vegliante[1], Maria Monica Barzago[1], Arianna Piotti[1], Roberto Chiesa[1], Giuseppe Di Fede[2], Fabrizio Tagliavini[2], Marco Gobbi[1], Mario Salmona[1], Elisa Zanier[1]

[1] *Department of Molecular Biochemistry and Pharmacology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy*
[2] *Neurology 5/Neuropathology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy.*

Tauopathies are a group of neurodegenerative disorders caused by the presence in different brain areas of insoluble and hyperphosphorylated tau deposits, alone or in association with a second aggregated protein. No effective pharmacological treatments are available for these diseases, which have high social and economic costs. Understanding the molecular mechanisms involved in disease onset and progression is a priority for developing strategies to prevent or postpone their incidence and mitigate the disease course.
We established an integrated approach involving biochemical and biophysical studies, based on the use of the invertebrate nematode *C. elegans* and mice models of tauopathy (i.e., transgenic mice expressing P301L-mutated tau and traumatic brain-injured mice) to evaluate the biological relevance of the different structural tau assemblies *in vivo*. We particularly focused on the formation of soluble tau oligomers, very reactive intermediates formed during the protein aggregation process, which are more toxic than the fibrillar species.
Neuronal functions and genes encoding enzymes to produce the neurotransmitters involved in synaptic transmission are conserved between *C. elegans* and vertebrates. In particular, the nematode's locomotion and pharyngeal activity are controlled by acetylcholine receptors. Thus *C. elegans* can be used as a "biosensor" able to specifically detect toxic tau forms in the brain with tauopathy.
This approach allowed us to quickly and cost-effectively investigate the mechanisms underlining tau toxicity and test pharmacological agents that interfere with tauopathy.

PHARMACOLOGICAL TARGETS FOR NEUROPROTECTION

Development of pharmacological chaperones targeting alpha synuclein neurotoxicity in preclinical models of Parkinson's disease

Alessio Masi[1], Antonino Iurato-La Rocca[1], Giuseppe Ranieri[1], Guido Mannaioni[1], Francesca Clemente[2], Camilla Matassini[2], Francesca Cardona[2], Andrea Goti[2].

[1] *Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino – NEUROFARBA, Università degli Studi di Firenze.*
[2] *Dipartimento di Chimica "Ugo Schiff" – DICUS, Università degli Studi di Firenze.*

The clinical manifestations of Parkinson's Disease (PD) are caused by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), a deep brain structure involved in the control of voluntary movement. Although the pathophysiology of PD is still incompletely understood, there is wide consensus about the role of insoluble alpha-synuclein (αSyn) aggregates in promoting progressive neuronal loss. In recent years, the pathological loop between αSyn and the lysosomal enzyme Glucocerebrosidase (GCase) has been implicated in the increased risk of developing PD in subjects with Gaucher Disease, a rare lysosomal disorder caused by homozygous GCase mutations. Since defective GCase activity has also been reported in sporadic PD, functional GCase enhancers such as pharmacological chaperones (PCs) may represent a useful therapeutic approach in a large proportion of PD patients. PCs are orally available small molecules that can access the Central Nervous System (CNS), bind and stabilize their target protein and thus increase GCase activity in the brain. We first determined the safety and the pharmacokinetic parameters of the candidate PC CF30 administered orally, and studied the target engagement in cerebral tissue with an enzymatic activity assay. We then established a mouse model of PD based on the AAV-mediated overexpression of the pro-aggregation human αSyn A53T mutation in the nigrostriatal pathway. On this model, we tested the efficacy of CF30 to prevent the deposition of αSyn aggregates in the striatum and SNc by means of immunohistochemical analysis.

BACE1: a potential biomarker for Alzheimer's disease therapy

Carlo Cervellati

Department of Translational Medicine and for Romagna, University of Ferrara (Ferrara, Italy)

β-Secretase 1 (BACE1) is central in the metabolism of amyloid β (Aβ) and formation of Alzheimer's disease (AD)-related senile plaques, since it catalyzes the rate-limiting step of amyloidogenic pathway. Despite the recent failure of clinical trials, BACE1 Inhibitors remain among the most advanced drug candidates for AD. Converging preclinical and *postmortem* evidence showed that BACE1 increases in AD brain. We have recently demonstrated that this pattern reflects in the serum of patients with mild cognitive impairment (MCI) and AD, with both presenting more than 30% higher activity compared to cognitively normal individuals ($p<0.001$ for both comparisons, total sample, n=918). The accuracy for the diagnosis of MCI and AD was around 80%. Notably, BACE1 activity was found to be increased in amnestic MCI patients converting to dementia compared to stable MCI, but only within a subsample with a less compromised cognitive status ($p<0.05$, median follow-up= 3 years). In conclusion, our findings suggest that BACE1 up-regulation might be one of the precocious aberration occurring in AD. In these perspectives, the evaluation of its serum activity might be useful in clinical trials as tool for screening patients for eligibility and monitoring the responses to the treatment.

Resilience to chronic stress is promoted by the coupling of RACK1 with the beta isoform of the glucocorticoid receptor

Erica Buoso[1], Paola Brivio [2], Mirco Masi [1,3], Maria Teresa Gallo [1], Piotr Gruca d , Magdalena Lason [4] , Ewa Litwa [4], Mariusz Papp [4] , Fabio Fumagalli [2] , Marco Racchi [1] , Emanuela Corsini [5] , Francesca Calabrese [2]

[1] *Dipartimento di Scienze del Farmaco, Università Degli Studi di Pavia, Pavia, Italy*
[2] *Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy*
[3] *Scuola Universitaria Superiore IUSS, Pavia, Italy*
[4] *Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland*
[5] *Dipartimento di Scienze e Politiche Ambientali, Università Degli Studi di Milano, Milan, Italy*

Vulnerability or resilience to chronic stress are mediated by several intracellular pathways thus contributing to maladaptation or adaptation to environmental challenges, respectively. The activity of the hypothalamic-pituitary-adrenal (HPA) axis is essential for the maintenance of brain processes, and it is related to the functionality of its primary regulator, the glucocorticoid receptor (GR), specifically alfa and beta isoforms. Among downstream effectors of the GR, the scaffolding protein RACK1 displays an important role in regulating synaptic activity and mediates the transcription of the neurotrophin BDNF.
Our results, obtained and confirmed both *in vitro* and *in vivo*, demonstrate that resilience to two weeks of chronic mild stress (CMS) is paralleled by the activation of GRβ-RACK1-BDNF pathway in the ventral hippocampus, which is the hippocampal subregion involved in the modulation of stress response. Hence, these data provide new critical information about the resilience to chronic stress and to the link among GR, RACK1 and BNDF, thus suggesting novel targets for the treatment of stress-related disorders, including depression.

PROTECTIVE EFFECTS OF THE MITOCHONDRIA-TARGETED ANTIOXIDANT ANTIOXCIN4 AGAINST OXIDATIVE STRESS IN THE AMYOTROPHIC LATERAL SCLEROSIS *SOD1^{G93A}* MOUSE

Débora Mena[1,2,3*], Pedro Soares[4], Fernando Cagide[4], Sofia Benfeito[4], Luís Grilo[1,2,3], Daniela F. Silva[1], Pedro Valente[1], Paulo Pinheiro[1], Elisabete Ferreiro[1,2], José Teixeira[1,2], Filomena Silva[1], Fernanda Borges[4], Paulo J. Oliveira[1,2,3], Ana I. Duarte[1,2,3]

[1] *CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal;*
[2] *CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal;*
[3] *Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal;*
[4] *CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal.*

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by motor neuronal loss, muscle atrophy, paralysis and death. ALS pathophysiology remains elusive, but mitochondrial dysfunction and oxidative stress may constitute pivotal mechanisms. Hence, recovering mitochondrial function and/or antioxidant defenses may delay ALS progression. We hypothesized that the mitochondria-targeted antioxidant AntiOxCIN4 can mitigate ALS severity by counteracting oxidative stress in the brain and skeletal muscle of ALS SOD1^{G93A} mice.

SOD1^{G93A} ALS mice were subcutaneously injected with 0.1 mg/Kg/day AntiOxCIN4, for 2 months. Oxidative/nitrosative stress markers, carbonyls, nitrites, hydroperoxides, and SOD1, SOD2 and glutathione reductase activities were measured in brain cortical and skeletal muscle tissue homogenates.

We observed that AntiOxCIN4 reduced nitrites and carbonyls' levels in ALS mouse brain (by 90% and 21%, respectively) and skeletal muscle (by 91% and 28%, respectively). AntiOxCIN4 also slightly diminished brain hydroperoxides (by 42%), and upregulated SOD1, SOD2 and glutathione reductase activities (by 696%, 112% and 109%, respectively) in ALS mice.

In sum, our very preliminary results suggest that peripherally administered AntiOxCIN4 may partially mitigate brain and skeletal muscle oxidative/nitrosative stress, ultimately delaying ALS progression. Further studies are required to strengthen our conclusions.

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A Lipidomic Study to Investigate Aß$_{1-42}$ Toxic Effects in SH-SY5Y Cells and Highlight New Targets for Alzheimer's Disease Drug Discovery

Lara Davani,[1] Xiaoqing Fu,[2] Angela De Simone,[3] Peng Li,[2] Serena Montanari,[1] Michael Lämmerhofer,[2] and Vincenza Andrisano[1]

[1] *Department for Life Quality Studies, University of Bologna, Corso D' Augusto 237, 47921 Rimini, Italy;*
[2] *Institute of Pharmaceutical Sciences, University of Tuebingen, Auf der Morgenstelle 8, 72076 Tuebingen, Germany;*
[3] *Department of Drug Science and Technology University of Torino, via P.Giuria 9, 10125 Torino, Italy.*

Currently not all Alzheimer's Disease (AD) pathological pathways, which lead to cell death and dementia, are completely investigated; among these, the lipid changes in brain tissues that begin years before AD symptoms [1]. While the central role of amyloid aggregation process is well established [2][3], more in-depth studies must be conducted to investigate the connections of this process to the alterations in brain lipid metabolism. Therefore, we aimed at developing a lipidomic approach to evaluate the Aß$_{1-42}$ toxic effects on differentiated human neuroblastoma derived SH-SY5Y cells. Cells were treated with increasing concentration of Aß$_{1-42}$ at different times, then the lipid extraction was carried out with IPA: H2O (90:10 v/v). LC-MS analysis of samples was performed by RP-UHPLC system coupled with a high-resolution quadrupole TOF mass spectrometer in comprehensive data-independent SWATH acquisition mode. Data processing was achieved via MS-DIAL (version 4.24) [4][5]. Each lipid class profiling in SH-SY5Y cells treated with Aß$_{1-42}$ was compared to the one obtained for the untreated cells to identify and relatively quantify some altered species in various lipid classes. This approach was found suitable to underline some peculiar lipid alterations, suitable as biomarkers, that might be correlated to Aß$_{1-42}$ different aggregation species and to deeply explore the cellular response mechanisms to the toxic stimuli [6][7]. The present method can also be applied to the elucidation of amyloid aggregation inhibitors mechanism of action, able not only to inhibit the aggregation process, but also to reduce lipid alterations in view of AD new drugs discovery [8].

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NEUROGLOBIN AS AN INTERCELLULAR TRASMITTER OF NEUROPROTECTION

Virginia Solar Fernández, Marco Fiocchetti, Maria Marino

Department of Science, Roma Tre University, Viale Guglielmo Marconi 446, I-00146 Rome, Italy

The compensatory protein Neuroglobin (NGB) displays, when overexpressed, intracellular cytoprotective functions against cellular insults (e.g., hypoxia, oxidative stress) and neurodegenerative conditions. We previously defined the critical role of Estrogen Receptor β (ERβ) signaling activation by 17β-estradiol (E2) or exogenous ligands (e.g., polyphenol Resveratrol-Res) in the up-regulation of NGB levels that drive cells to anti-apoptotic responses. Recent evidence suggested an extracellular NGB release, raising the possibility of novel neuroprotective mechanisms of the globin outside cells. Here we investigated the effects of NGB inducers on the globin release in neuronal cells, assessing the functionality of the secreted protein. Data indicate that NGB is secreted by neuronal derived cells SH-SY-5Y through the exosomal and non-exosomal mechanisms following the activation of the estradiol pathway and under oxidative stress (H_2O_2) conditions. Furthermore, for the first time, we demonstrate that NGB-enriched condition media (CM), collected by NGB overexpressing cells, prevent the early mitochondrial fragmentation and, in turn, reduce apoptotic cell death in SH-SY-5Y cells after oxidative stress or 3-nitropropionic acid (3NP) treatment. Of note, a similar anti-apoptotic effect was obtained by using the NGB-enriched exosomal or the exosome-deprived CM fractions suggesting that extracellular NGB released as a free protein or through extracellular vesicles can be neuroprotective independently. Altogether, reported results strengthen the idea that NGB effects are not limited to the cell compartment being the globin able to function as a transmission factor of neuronal cell resilience pointing NGB as a possible targetable neurotrophic protein in neurodegenerative disease.

Targeting GSK-3β for Neuroprotection in Angiotensin II-treated cells

Derviş Birim[1], Güliz Armagan[1]

Department of Biochemistry, Faculty of Pharmacy, Ege University, İzmir, Turkey[1]

Glycogen synthase kinase (GSK)-3β plays a significant role in physiological and pathological conditions. It has been reported that the overactivation of this enzyme is closely related to neuroinflammation. Angiotensin II (Ang II) is also known to induce proinflammatory cytokine production in the brain. In this study, we aimed to investigate whether GSK-3β is activated when cortical neuronal cells are stimulated with Ang II. For this reason, we treated human cortical neuronal cell line (HCN-2) with Ang II (0.1-50 μM) for 6 or 24h. Cell viability and phosphorylated forms of GSK3β (active and inhibited) were evaluated by WST-1 and Western Blotting assays, respectively. Selected concentrations of Ang II did not significantly alter cellular viability. However, significant increase in pGSK-3β(Tyr216) (active form) and decrease in pGSK-3β(Ser9) (inhibited form) levels were observed following 10 μM Ang II treatment for 24 h ($p<0.05$). It can be concluded that GSK3β is regulated by Ang II in cortical neuronal cells. Newly synthesized inhibitors can be used to reverse Ang II induced GSK3β overactivation and ultimately protect neuronal cells.

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Dysregulation of long non-coding RNAs in neuroinflammation: characterization of *LINC00520* and its possible role in Parkinson's disease

Martina Fazzina[1], Letizia Pruccoli[1], Andrea Tarozzi[1], Francesca Massenzio[3], Barbara Monti[3], Davide Maestri[3,4], Matteo Bergonzoni[2], Flavia Frabetti[2] and Raffaella Casadei[1]

[1] *Department for Life Quality Studies, University of Bologna, Rimini, Italy*
[2] *Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy*
[3] *Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy*
[4] *The Wistar Institute, Philadelphia, Pennsylvania, USA.*

Parkinson's disease (PD) is a widely spread neurodegenerative disorder caused by both genetic and environmental factors, leading to the appearance of characteristic motor and non-motor symptoms. Activated microglia plays a pivotal role in the pathogenesis of PD, sustaining inflammatory processes at the level of neurodegenerative regions by releasing Reactive Oxygen and Nitrogen Species, exposing neurons to oxidative stress. It has recently been observed that the dysregulation of long non-coding RNAs (lncRNAs) could affect the modulation of microglial phenotype in neurodegenerative diseases. Interestingly, we identified a differential expression of some lncRNAs by comparing transcriptomic data of PD patient's *vs* control brain samples through a detailed meta-analysis. Our study aims to characterize the expression and function of *LINC00520*, a lncRNA not previously linked to PD, in several PD models. In particular, we tested its gene expression compared to a control lncRNA, *LINC00674,* IN human neuroblastoma SH-SY5Y cells subjected to 6-OHDA treatment and in human monocytic THP-1 cells differentiated towards microglial fate with PMA and stimulated with LPS. We then enrolled the human microglia cell line hmc3 treated with ifn-γ boosted by glucose, to promote the NF-κB inflammatory pathway, or 6-OHDA. The results show a significant upregulation of *LINC00520* both in inflammatory states and in oxidative stress conditions. Moreover, preliminary data of RNAi assays against *LINC00520* suggested its involvement in oxidative stress-related pathways. Characterization of *ZFLNCG09760*, a putative *LINC00520* ortholog in zebrafish, as an *in vivo* model, is currently ongoing.

A novel approach on pain treatment

Nicoletta Marchesi[1,2]

[1] *University of Pavia, Department of Drug Sciences and NeuHeart srl*
[2] *N. Marchesi was recipient of a fellowship by NeuHeart srl*

Globally, there are important unmet medical needs in the field of pain therapy. The sensation of pain involves communication between nerves, spinal cord, and brain. Given the growing number of patients suffering from pain not responding to therapy, there is an urgent need of discovering new analgesic agents as well as new strategies to tackle pain development. Particularly, strong evidence indicates that substances antagonizing the NGF receptor TrkA can act as analgesics both against acute and chronic pain. Moreover, these substances may be effective in preventing chronic pain development following acute pain due to surgical procedures. However, no drugs have so far been approved in pain therapy acting through this mechanism, and there is concern among researchers on the possible consequences on neuroprotection. Within this context, data obtained during an *in silico* characterization of a specific region of interest of the TrkA receptor, containing two cysteines originating a disulphide bond, highlighted that these residues are important in order to maintain the three-dimensional structure of TrkA when associating with NGF. Specifically, *in vitro* experiments indicated that interfering with this disulphide bond by reducing agents (i.e. DTT, NAC both at millimolar concentrations) reduced but did not abolished *in vitro* NGF stimulated TrkA activity and decreased pain sensation using *in vivo* models of pain in mice treated with formalin in the hind paw and intrathecally with NAC (12 mg/Kg in 5 μl). The results and literature data on NAC suggest a novel model of balanced TrkA inhibition modulating pain without compromising neuroprotection.

Sex-dependent effects of CYP46A1 overexpression on cognitive function during aging

María Latorre-Leal[1], Patricia Rodriguez-Rodriguez[1], Luca Franchini[1,2], Makrina Daniilidou[1,3], Francesca Eroli[1], Bengt Winblad[1] , Kaj Blennow [4,5], Henrik Zetterberg [4,5] , Miia Kivipelto[1,6], Manuela Pacciarini[7], Yuqin Wang[7], William J. Griffiths[7], Ingemar Björkhem[8], Anna Sandebring Matton [1,3], Paula Merino-Serrais[1,9,10], Angel Cedazo-Minguez[1], **Silvia Maioli[1]**

[1] *Karolinska Institutet, Department of Neurobiology Care Sciences and Society, Division of Neurogeriatrics, Center for Alzheimer Research, Stockholm, Sweden.*
[2] *DiSFeB, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, via Balzaretti 9, 20133 Milano, Italy.*
[3] *Karolinska Institutet, Department of Neurobiology Care Sciences and Society, Division of Clinical Geriatrics, Center for Alzheimer Research, Stockholm, Sweden.*
[4] *Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.*
[5] *Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.*
[6] *Theme Aging, Karolinska University Hospital, Stockholm, Sweden*
[7] *Swansea University Medical School, SA2 8PP Swansea, United Kingdom.*
[8] *Department of Laboratory Medicine, Karolinska Institute, Huddinge, Sweden.*
[9] *Laboratorio Cajal de Circuitos Corticales (CTB), Universidad Politécnica de Madrid, Madrid, Spain.*
[10] *Departamento de Neurobiología Funcional y de Sistemas, Instituto Cajal, CSIC, Madrid, Spain*

Cholesterol turnover and CYP46A1 regulation are reported to be crucial for memory functions. An increasing body of evidence shows that CYP46A1 activation is able to reduce Alzheimer's Disease (AD) pathological processes. In this study we report for the first time that CYP46A1 overexpression and increase of 24S-hydroxycholesterol (24OH) induces sex-specific changes in synaptic functions in aged mice, being beneficial in females while detrimental in males. The positive effects on cognition in aged CYP46A1 overexpressing female mice were accompanied by morphological changes in dendritic spines and enhancement of estrogen receptor signaling in hippocampus. In aged males, CYP46A1 overexpression leads to anxiety-like behavior and worsening of spatial memory, followed by decreased dendritic spine density and higher 5a-dihydrotestosterone (DHT) levels in hippocampus. Further, analysis of cerebrospinal fluid (CSF) from AD, mild cognitive impairment and healthy patients revealed that 24OH was negatively associated to markers of neurodegeneration in women but not in men. Based on our results, CYP46A1 activation may represent a pharmacological target that could specifically enhance brain estrogen receptor signaling in women at risk of developing AD. Finally, this study highlights the importance of taking into account the sex-dimension in both preclinical and clinical studies of neurodegenerative diseases like AD.

DIETARY SUPPLEMENTS, NUTRACEUTICALS, AND FUNCTIONAL FOODS IN NEUROPROTECTION

Antioxidants salvage from Zinc-induced neurotoxicity via mitigation of oxidative stress, dopamine transporters dyshomeostasis and neuronal apoptosis

Garima Singh[1,2] and Chetna Singh[1,2]

[1] *Developmental Toxicology Laboratory, Systems Toxicology and Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Vishvigyan Bhawan, 31, Mahatma Gandhi Marg, Lucknow 226 001, Uttar Pradesh, India.*

[2] *Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201 002, Uttar Pradesh, India*

Brain Zinc (Zn) pool is crucial for the proper neuronal functions, however; excessive Zn levels are associated with neurological disorders including Parkinson's disease (PD). Oxidative stress is recognized as the forerunner responsible for the manifestations in Zn-induced neurotoxicity. The present study explored the protective role of synthetic superoxide dismutase/SOD mimetic (tempol) and natural antioxidant (silymarin) against Zn-induced dopaminergic neuronal degeneration. Animals were treated with $ZnSO_4$ (20 mg/kg; i.p.), twice weekly with or without Silymarin/Tempol for 12 weeks along with respective controls. Zn exposure caused striatal dopamine decline with simultaneous reduction in the protein levels of dopamine synthesizing enzyme tyrosine hydroxylase/TH. Zn elevated lipid peroxidation levels and SOD activity while glutathione/GSH and GSH-S-transferase/GST activity were diminished. In addition, the expression of dopamine transporter/DAT and mitochondrial cytochrome c release were augmented with concurrent decrease in the levels of vesicular monoamine transporter-2/VMAT-2 and pro-caspase 3/9 in Zn exposed groups. Tempol and silymarin protected against Zn-induced dopamine/TH diminution and oxidative stress. Furthermore, tempol and silymarin also restored the levels of DAT, VMAT-2, mitochondrial cytochrome c and pro-caspase 3/9 in Zn exposed groups. The results of the study demonstrate that tempol/silymarin alleviated Zn-induced oxidative stress leading to restoration of monoamine transporters and prevention of cellular apoptosis thereby rescuing from Zn-induced neurotoxicity.

Hidrox® Roles in Neuroprotection: Biochemical Links between Traumatic Brain Injury and Alzheimer's Disease

Ramona D'Amico[1], Rosalba Siracusa[1], Roberta Fusco[1], Marika Cordaro[2], Angela Trovato Salinaro[3], Daniela Impellizzeri[1], Maria Scuto[3], Maria Laura Ontario[3], Roberto Crea[4], Salvatore Cuzzocrea[1], Rosanna Di Paola[1], Vittorio Calabrese[3]

[1] *Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98166 Messina, Italy;*
[2] *Department of Biomedical, Dental and Morphological and Functional Imaging University of Messina, Via Consolare Valeria, 98125 Messina, Italy;*
[3] *Department of Biomedical and Biotechnological Sciences, University of Catania, 95124 Catania, Italy;*
[4] *Oliphenol LLC., 26225 Eden Landing Road, Unit C, Hayward, CA 94545, USA.*

Traumatic brain injuries (TBI) are a serious public-health problem. Furthermore, subsequent TBI events can compromise TBI patients' quality of life. TBI is linked to a number of long- and short-term complications such as cerebral atrophy and risk of developing dementia and Alzheimer's Disease (AD). Following direct TBI damage, oxidative stress and the inflammatory response lead to tissue injury-associated neurodegenerative processes that are characteristic of TBI-induced secondary damage. Hidrox® showed positive effects in preclinical models of toxic oxidative stress and neuroinflammation; thus, the aim of this study was to evaluate the effect of Hidrox® administration on TBI-induced secondary injury and on the propagation of the AD-like neuropathology. Hidrox® treatment reduced histological damage after controlled cortical impact. Form a molecular point of view, hydroxytyrosol is able to preserve the cellular redox balance and protein homeostasis by activating the Nrf2 pathway and increasing the expression of phase II detoxifying enzymes such as HO-1, SOD, Catalase, and GSH, thus counteracting the neurodegenerative damage. Additionally, Hidrox® showed anti-inflammatory effects by reducing the activation of the NFkB pathway and related cytokines overexpression. From a behavioral point of view, Hidrox® treatment ameliorated the cognitive dysfunction and memory impairment induced by TBI. Additionally, Hidrox® was associated with a significant increased number of hippocampal neurons in the CA3 region, which were reduced post-TBI. In particular, Hidrox® decreased AD-like phenotypic markers such as ß-amyloid accumulation and APP and p-Tau overexpression. These findings indicate that Hidrox® could be a valuable treatment for TBI-induced secondary injury and AD-like pathological features.

Identification of neuroprotective natural compounds acting via mitochondria hormesis: *C. elegans* as a unique multicellular and preclinical model organism for screening and intervention studies

Silvia Maglioni *[,1], Alfonso Schiavi [1], Nayna Arsalan [1], Shiwa Afshar[1], Joel N Meyer [2], Natascia Ventura [1,3]

[1] *IUF-Leibniz Research Institute for Environmental Medicine, 40225 Duesseldorf, Germany*
[2] *Nicholas School of the Environment, Duke University, Durham, NC, United States*
[3] *Institute for Clinical Chemistry and Laboratory Diagnostic, Medical Faculty of the Heinrich Heine University, 40225 Duesseldorf, Germany*

Severe mitochondria dysfunction is causally linked to variety of neuropathologies ranging from developmental to age-associated disorders. Yet, interestingly, mild mitochondrial stress promotes health-span (mitohormesis) across species. The nematode *Caenorhabditis elegans* is a powerful multicellular model organism for *in vivo* preclinical and interventions studies, which we primarily employ in our lab to identify mitochondrial stress responses (MSR) underlying the beneficial effects of mitohormesis [1-3]. We hypothesized that compounds inducing MSR may have neuroprotective effects in both developmental and age-associated diseases.

To identify such compounds, we exploited the very reproducible, discrete and automatically quantifiable *C. elegans* features, ensuing from genetic- or chemical-induced mitochondria hormesis, to develop a high-content microscopy screening platform [4]. We recently used this platform to screen a small library of natural compounds on the one hand in search of anti-aging interventions and on the other hand in *C. elegans* models of mitochondriopathies in search of disease suppressors.

Strikingly, and in strong support of our hypothesis, the two independent screens lead to the identification of the same 4 compounds, which we found display neuroprotective effects at different concentrations in a mitochondria disease model and during aging. We focused on the most promising hit and showed that Lutein promotes healthy aging: increases animals' resistance to heat shock, extends lifespan and promote locomotion ability. Moreover, Lutein suppresses the neurodevelopmental defects we characterized in different mitochondriopathies models. Our findings indicate natural compounds identified in our screen promote neuronal health via mitochondrial hormesis.

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Early treatment with nutraceuticals and drugs for the rescue of intellectual disability in Down syndrome

Fiorenza Stagni[1], Marco Emili[2], Sandra Guidi[2], Giorgio Aicardi[1], Renata Bartesaghi[2]

[1] *Department for Life Quality Studies, University of Bologna, Italy*
[2] *Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy*

Down syndrome (DS) is a relatively high-incidence genetic condition due to triplication of chromosome 21. Intellectual disability (ID) represents the most invalidating aspect of this pathology and is mainly attributable to neurogenesis and dendritogenesis alterations that can be traced back to the earliest neurodevelopmental phases.
No therapies currently exist for ID in DS. The overall goal of our studies is to identify therapies that can reinstate brain development and, hopefully, ID in DS. To this purpose, we exploit the Ts65Dn mouse model that recapitulates many anatomical/functional alterations of DS. Considering the critical time windows for brain development, we treat mice during the prenatal or early postnatal period with selected compounds, including nutraceuticals, that are known to foster neurogenesis or target pathways that are deranged in DS. By using multiple approaches, we establish the effect of the administered therapies on neurogenesis, cellularity, neuronal maturation, connectivity, synaptic plasticity, and cognitive performance. Synaptic plasticity, a key mechanism underlying memory, is known to be impaired in the hippocampal formation of Ts65Dn mice. Importantly, we could demonstrate through electrophysiological recordings that it is also impaired in the perirhinal cortex, a key region for visual recognition memory, that is typically altered in DS.
Thanks to these complementary approaches, we found that some of the attempted therapies are very effective in restoring trisomy-linked neurodevelopmental defects and cognitive performance in the Ts65Dn model. In view of the good translational impact of some of these therapies, we hope that our findings will prompt clinical trials in children with DS.

SCFA Treatment Alleviates Pathological Signs of Migraine and Related Intestinal Alterations in a Mouse Model of NTG-Induced Migraine.

Marika Lanza[1], Alessia Filippone[1], Alessio Ardizzone[1], Giovanna Casili[1], Irene Paterniti[1], Emanuela Esposito[1] and Michela Campolo[1]

[1] *Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno D'Alcontres, 31-98166 Messina, Italy*

Background: There is a growing realization that the gut–brain axis signaling is critical for maintaining the health and homeostasis of the Central Nervous System (CNS) and the intestinal environment. The role of Short-Chain Fatty Acids (SCFAs), such as Sodium Propionate (SP) and Sodium Butyrate (SB), has been reported to counteract inflammation activation in the central and Enteric Nervous System (ENS). Methods: In this study, we evaluated the role of the SCFAs in regulating the pathophysiology of migraine and correlated dysregulations in the gut environment in a mouse model of Nitroglycerine (NTG)-induced migraine. Results: We showed that, following behavioral tests evaluating pain and photophobia, the SP and SB treatments attenuated pain attacks provoked by NTG. Moreover, treatments with both SCFAs reduced histological damage in the trigeminal nerve nucleus and decreased the expression of proinflammatory mediators. Ileum evaluation following NTG injection reported that SCFA treatments importantly restored intestinal mucosa alterations, as well as the release of neurotransmitters in the ENS. Conclusions: Taken together, these results provide evidence that SCFAs exert powerful effects, preventing inflammation through the gut–brain axis, suggesting a new insight into the potential application of SCFAs as novel supportive therapies for migraine and correlated intestinal alterations.

SCIENTIFIC SESSION 4

DRUG DISCOVERY IN NEURODEGENERATIVE DISEASES

L-Dopa-containing diketopiperazines as potential anti-Parkinson agents

Ivana Cacciatore

Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, Italy

Parkinson's Disease (PD) is a neurodegenerative disorder of the central nervous system (CNS) characterized by motor dysfunctions, such as bradykinesia, rigidity, neuropsychiatric symptoms, and others. The pharmacological treatment of the disease is only symptomatic since, to date, there is no treatment to stop or slow PD. Currently, L-Dopa (LD) remains the gold standard therapy but its poor bioavailability (low water and lipid solubility, high susceptibility to chemical and enzymatic degradation) reduces the pharmacological effect.
Delivery of most drugs into the CNS is restricted by the blood brain barrier (BBB), which remains a significant bottleneck for the development of novel CNS-targeted therapeutics. During last decades different medicinal chemistry-based approaches were developed to improve pharmaceutical, pharmacokinetic, and pharmacodynamic properties of hydrophilic compounds, such as LD. The diketopiperazine (DKP)-based motif was recently considered as potential blood-brain barrier shuttle (BBB-shuttle) for the delivery of drugs with limited ability to cross the BBB. Starting from this consideration, novel LD-containing diketopiperazines were developed in our laboratories. The peculiar heterocyclic scaffold of DKPs confers high stability against the proteolysis and constitutes a structural requirement for increased cell permeability, higher activity and selectivity, and less cytotoxicity. Moreover, DKPs can cross the blood–brain barrier via passive diffusion process representing an exciting challenge for medicinal chemists with the aim of improving the pharmacological efficacy of novel drugs.

Design of nanomolar selective type-II inhibitors of glycogen synthase kinase-3β with promising neuroprotective effects in a cellular model of Alzheimer's disease

Joseph M. Hayes[1], Matthew P Davies[1], Rocio Benitez[2], Concepcion Perez[2], Sven Jakupovic[3], Philip Welsby[1], Klaudia Rzepecka[1], Jane Alder[1], Colin Davidson[1], Ana Martinez[2]

[1] *School of Pharmacy & Biomedical Sciences, University of Central Lancashire, Preston PR1 2HE, United Kingdom.*
[2] *Centro de Investigaciones Biologicas, CSIC, 28040 Madrid, Spain.*
[3] *AnalytiCon Discovery GmbH, 14473 Potsdam, Germany.*

Glycogen synthase kinase – 3β (GSK-3β) is an important target for new Alzheimer's disease treatments [1]. The enzyme is phylogenetically related to other kinases making the design of *both* potent and selective type-I (ATP-binding site) inhibitors difficult, with the Asp-Phe-Gly (DFG) motif of the activation loop orientated toward the binding site (DFG-in, active conformation). On the other hand, design of inhibitors targeting the DFG-out inactive conformation (type-II inhibitors) has the potential to lead to more selective inhibitors, exploiting additional interactions in the hydrophobic allosteric site immediately adjacent to the ATP-binding site. However, this has not been previously actively pursued for GSK-3β. In this study, we present the successful *in silico* screening and *in vitro* validation of potent selective GSK-3β type-II inhibitors [2]. In the absence of crystallographic evidence for a DFG-out GSK-3β conformation, a computational model was constructed using Prime loop refinement, induced-fit docking, and molecular dynamics. Virtual screening led to an initial selection of 20 Phase I compounds, yielding two low micromolar GSK-3β inhibitors. Twenty analogues (Phase II compounds) related to the hit [pyrimidin-2-yl]amino–furo[3,2-b]furyl–urea scaffold were selected for structure–activity relationship analysis. These Phase II studies led to five highly potent GSK-3β nanomolar inhibitors, the best compound (IC_{50} = 0.087 μM) > 100 times more potent than the best Phase I inhibitor. Selectivity for GSK-3β inhibition compared to homologous kinases was observed. *Ex vivo* experiments (SH-SY5Y cell lines) for tau hyperphosphorylation revealed promising neuroprotective effects, with compounds out-performing TDZD-8 at low micromolar concentrations.

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Seril-18, a new synthetic molecule, inhibits α-synuclein aggregation in an experimental model of Parkinson's disease

Roberta Fusco[1], Rosalba Siracusa[1], Marika Cordaro[2], Ramona D'Amico[1], Daniela Impellizzeri[1], Rosanna Di Paola[1]

[1] *Department of Chemical, Biological, Pharmaceutical and Environmental Science, University of Messina, Messina, Italy;*
[2] *Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy;*

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a gradual damage of dopaminergic neurons in the substantia nigra and presence of α-synuclein (α-syn)-rich cytoplasmic neuronal inclusions named Lewy bodies. Currently, the pharmacological management of PD is limited only to addressing the symptomatic features, not the progression of the disease. At this regard, we explore the neuroprotective effect of Seril-18, a new synthetic molecule that acts as a selective inhibitor of α-syn aggregation, in an experimental model of PD. PD was induced in mice by four intraperitoneal injections of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at the dose of 20 mg/kg every 2 h for a total administration of 80/kg, and Seril-18 (10 mg/kg) was administered by oral gavage for 7 days.
Seril-18 significantly reduced α-synuclein aggregation in neurons, leading to a decrease in behavioral impairments and neuronal cell degeneration of the dopaminergic tract. Moreover, Seril-18 treatment was able to increased tyrosine hydroxylase and dopamine transporter activities, and prevented dopamine depletion. Seril-18 also modulated nuclear factor-κB pathway, as well as modulated the activation of astrocytes and microglia.
In conclusion, these findings propose this new compound as a valid approach to prevent neurodegeneration and neuroinflammation associated with PD.

A new molecular strategy at CB2 Receptor: from orthosteric and allosteric modulators to dualsteric/bitopic ligands

Francesca Gado[1,2], Rebecca Ferrisi[2], Beatrice Polini[1], Kawthar A. Mohamed[3], Elena Lucarini[4], Sara Carpi[1], Lesley A. Stevenson[5], Caterina Ricardi[1], Roger G. Pertwee[5], Paola Nieri[2], Carla Ghelardini[4], Lorenzo Di Cesare Mannelli[4], Grazia Chiellini[1], Robert B. Laprairie[3], Clementina Manera[2].

[1] *Department of Pathology, University of Pisa, Pisa , Italy*
[2] *Department of Pharmacy, University of Pisa, Pisa, Italy*
[3] *College of Pharmacy and Nutrition, University of Saskatchewan, Saskatchewan, Canada*
[4] *Department of Neuroscience, Psychology, Drug Research and Child Health, Section of Pharmacology and Toxicology, University of Florence, Florence, Italy.*
[5] *School of Medicine, Medical Sciences and Nutrition, Institute of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, UK*

Cannabinoid receptor 2 (CB2R) up-regulation has been widely reported in animal models of Alzheimer's disease. However, so far selective CB2R ligands failed to succeed in the clinical trials on human patients because of the immune-suppression observed after chronic administration. Two strategies have been proposed to overcome this limitation while maintaining CB2R beneficial effects, namely the development of (i) CB2R positive allosteric modulators (CB2R-PAM), or (ii) CB2R bitopic/dualsteric ligands. Our group already reported the synthesis of **EC21a**, the first CB2R-PAM, which when tested in a murine microglia cells model of neuroinflammation (BV2 cell line), in combination with our previously developed **FM6b** full agonist, revealed to significantly increase the anti-inflammatory activity of the parent compound. These results opened the way to the design of novel CB2R bitopic/dualsteric ligands, obtained by linking the primary pharmocofore **FM6b** to the secondary pharmacofore **EC21a**, through chemically defined linkers. Newly developed compounds have been extensively tested in binding and functional assays. Analog **FD22a** emerged as the most promising CB2R bitopic/dualsteric ligand of the series and may require further investigation to confirm its potential as a new drug for the treatment and prevention of neurodegenerative diseases.

Multifunctional synthetic small molecules inspired by ferulic acid as potential anti-Alzheimer's disease agents

Lidia Ciccone[1], Beatrice Bargagna[2,3], Susanna Nencetti[1], M. Amélia Santos[2], Sílvia Chaves[2], Caterina Camodeca[1] and Elisabetta Orlandini [4]

[1] *Department of Pharmacy, University of Pisa, Via Bonanno 6, 56126 Pisa, Italy;*
[2] *Centro de Química Estrutural and Departamento de Engenharia Química, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal;*
[3] *Department of Earth Sciences, University of Pisa, Via Santa Maria 53-55, 56100 Pisa, Italy;*
[4] *Research Center "E. Piaggio", University of Pisa, 56122 Pisa, Italy*

Alzheimer's disease (AD) is a severe multifactorial neurodegenerative disorder characterized by a progressive loss of neurons in the brain. Early symptoms are memory decline and language problems, followed by other cognitive serious dysfunctions related to brain atrophy. Despite research efforts, the pathogenesis and mechanism of AD progression are not yet completely understood. There are only a few symptomatic drugs approved for the treatment of AD. The multifactorial character of AD suggests that it is important to develop molecules able to target simultaneously the several pathological mechanisms associated with the disease.
In the context of the worldwide recognized interest of multifunctional ligand therapy, we propose the synthesis, characterization, physicochemical and biological evaluation of five (**1a–e**) new ferulic acid-based hybrid compounds, namely feroyl-benzyloxyamidic derivatives enclosing different substituent groups, as potential anti-Alzheimer's disease agents. These hybrids can keep both the radical scavenging activity and metal chelation capacity of the naturally occurring ferulic acid scaffold, presenting also good/mild capacity for inhibition of self-Aß aggregation and fairly good inhibition of Cu-induced Aß aggregation. The predicted pharmacokinetic properties point towards good absorption, comparable to known oral drugs.

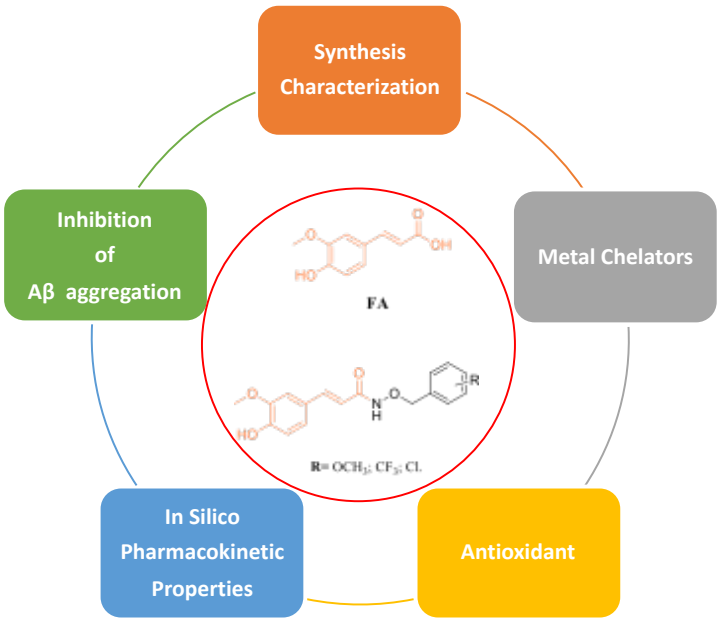

Figure 1. Graphical summary

SCIENTIFIC SESSION 5

DRUG DISCOVERY IN NEURODEGENERATIVE DISEASES

SUN11602, a novel bFGF mimetic, exerts neuroprotective effects in Parkinson's disease

Giovanna Casili[1], Alessio Ardizzone[1], Valentina Bova[1], Michela Campolo[1], Marika Lanza[1], Alessia Filippone[1], Emanuela Esposito[1], Irene Paterniti[1].

[1] *Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Ferdinando Stagno D'Alcontres, 31-98166 Messina, Italy.*

Parkinson's disease (PD) is the second most frequent neurodegenerative disease, with a large prevalence in industrialized countries. The etiopathogenesis of PD is multifactorial and not yet fully known, however, in the last few decades, scientific world advised the establishment of a neuroinflammatory state among the possible risk factors. In this field, basic fibroblast growth factor/fibroblast growth factor receptor 1 (bFGF/FGFR1) could be a promising way to treat CNS-mediated inflammation; however, the use of bFGF as a therapeutic agent is limited by its side effects.

The novel synthetic compound SUN11602 exhibited neuroprotective activities like bFGF, but demonstrating greater safety, thus constituting a potential candidate for the treatment of neurodegenerative diseases including PD. In this perspective, this study aimed to evaluate the effect of SUN11602 administration in a murine model of MPTP-induced PD. PD was induced by intraperitoneal injection of MPTP (80 mg/kg). SUN11602 (1, 2.5, and 5 mg/kg) was administered daily by oral gavage starting from 24 hours after the first administration of MPTP, mice were sacrificed 7 days after MPTP induction. The results obtained showed that SUN11602 administration significantly reduced the alteration of PD hallmarks, attenuating the neuroinflammatory state via modulation of glial activation, NF-κB pathway and cytokine overexpression. Furthermore, we demonstrated that SUN11602 treatment rebalanced Ca^{2+} overload in neurons by regulating Ca^{2+}-binding proteins, inhibiting the apoptotic cascade. Therefore, considering these findings, SUN11602 could be considered a valuable pharmacological strategy for PD.

Imidazolylacetophenone oxime ether T1 exhibits multifunctional neuroprotective effects and cognitive enhancement in 5xFAD Alzheimer's disease mice model

Jiang-Jiang Tang, Bo Ren, Lan-Fang Huang, Zhao-Yuan Bian, Jia-Le Deng

Shaanxi Key Laboratory of Natural Products & Chemical Biology, College of Chemistry & Pharmacy, Northwest A&F University, 3 Taicheng Road, Yangling, 712100, Shaanxi, P. R. China

Alzheimer's disease (AD) possesses a complex pathogenetic mechanism. Nowadays, multitarget agents are considered to have potential in effectively treating AD via triggering molecules in functionally complementary pathways at the same time. Here, based on the screening (~1400 compounds) against neuroinflammation, an imidazolylacetophenone oxime ether (**T1**) was discovered as a novel hit. **T1** exhibited potential multifunctional neuroprotective effects including anti-neuroinflammatory, antioxidative damage, metal-chelating, inhibition of acetylcholinesterase (AChE) properties. **T1** can dose-dependently suppress the expression of iNOS and COX-2 but not change the expression of HO-1 protein. Moreover, **T1** exhibited evidently neuroprotective effects on H_2O_2-induced PC12 cells damage and ferroptosis without cytotoxicity at 10 μM, as well as selectively metal chelating properties via chelating Cu^{2+}. In addition, **T1** showed a mixed-type inhibitory effect on AChE *in vitro*. Parallel artificial membrane permeation assay (PAMPA) also verified that **T1** can overcome the blood-brain barrier (BBB). To test effects of **T1** *in vivo*, 6-month-old 5xFAD mice were used as an AD model. **T1** (15 mg/kg B.W., *i.p.*) for 30 days attenuated the impairments in cognitive function observed in 5xFAD mice, as assessed with the Morris water maze, Y-maze, Barnes Maze tests. To determine the psychological behavioral changes in AD mice, the MBT and EPM were performed in the current study. We found that **T1** significantly elevated the entries onto the open arms in AD mice, which reveals preventive effects on anxiety-like behavior in the AD mice. Moreover, **T1** restored neuronal damage and postsynaptic density protein 95 (PSD95) expression in the hippocampus. To our best knowledge, this is the first report on imidazolylacetophenone oxime ether-based multifunctional neuroprotective effects and cognitive enhancement, suggesting that this type of compounds might be novel multifunctional agents against AD.

** E-mail: tangjiang11@nwafu.edu.cn (JJT);*

Antioxidant Activity of Fluoxetine and Vortioxetine in a Non-transgenic Animal Model of Alzheimer's Disease

Giuseppe Caruso[1], Margherita Grasso[1,2], Annamaria Fidilio[1,3], Sebastiano Alfio Torrisi[3], Nicolò Musso[3], Federica Geraci[3], Maria Rosaria Tropea[3], Anna Privitera[1], Fabio Tascedda[4,5], Daniela Puzzo[2,3], Salvatore Salomone[3], Filippo Drago[3], Gian Marco Leggio[3], Filippo Caraci[1,2]

[1] *Department of Drug and Health Sciences, University of Catania, Catania, Italy;*
[2] *Oasi Research Institute - IRCCS, Troina, Italy;*
[3] *Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy;*
[4] *Center for Neuroscience and Neurotechnology, University of Modena and Reggio Emilia, Modena, Italy;*
[5] *Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy.*

Depression is risk factor for the development of Alzheimer's disease (AD). A neurobiological and clinical continuum exists between AD and depression, with neuroinflammation and oxidative stress being involved in both diseases. Second-generation antidepressants, in particular selective serotonin reuptake inhibitors (SSRIs), are currently studied as neuroprotective drugs in AD. By employing a non-transgenic AD model, obtained by intracerebroventricular (i.c.v.) injection of amyloid-β (Aβ) oligomers in 2-month-old C57BL/6 mice, we recently demonstrated that the SSRI fluoxetine (FLX) and the multimodal antidepressant vortioxetine (VTX) reversed the depressive-like phenotype and memory deficits induced by Aβ oligomers rescuing the levels of transforming growth factor-β1 (TGF-β1). Aim of the present study was to test both FLX and VTX for their ability to prevent oxidative stress in the hippocampus of Aβ-injected mice, a brain area strongly affected in both depression and AD. The chronic intraperitoneal (i.p.) administration of FLX (10 mg/Kg) and VTX (5 and 10 mg/Kg) for 24 days, starting 7 days before Aβ injection, was able to prevent the over-expression of inducible nitric oxide synthase (iNOS) and NADPH oxidase 2 (Nox2) induced by Aβ oligomers. Antidepressant pre-treatment was also able to rescue the mRNA expression of glutathione peroxidase 1 (Gpx1) antioxidant enzyme. FLX and VTX also prevented Aβ-induced neurodegeneration in mixed neuronal cultures challenged with Aβ oligomers. Our data represent the first evidence that the chronic treatment with the antidepressants FLX or VTX can prevent the oxidative stress phenomena related to the cognitive deficits and depressive-like phenotype observed in a non-transgenic animal model of AD.

PQM130, a novel neuroprotective agent, to reduce the damage induced by β-amyloid$_{1-42}$ protein in a murine model of Alzheimer's disease

G. Sita[1], A.Graziosi[1], F.Morroni[1], A. Tarozzi[2], L. Pruccoli[2], C. Viegas Jr[3], P. Hrelia[1]

[1] *Alma Mater Studiorum – University of Bologna, Pharmacy and BioTechnology, Bologna, Italy*
[2] *Alma Mater Studiorum – University of Bologna, Life Quality Studies, Rimini, Italy*
[3] *Institute of Chemistry, Federal University of Alfenas, Alfenas, MG, Brazil*

Alzheimer's disease (AD) is the most frequent form of dementia in older people. Though AD etiology is still unknown, soluble amyloid species are recognized as fundamental for AD onset. To date, there are not effective therapeutic approaches able to interfere with AD's progression and donepezil, an acetylcholinesterase inhibitor, is the first-line acetylcholinesterase inhibitor used for the treatment.
In the present study, a novel feruloyl–donepezil hybrid compound (PQM130) was synthesized and evaluated as a multitarget drug candidate against the neurotoxicity induced by intracerebroventricular (i.c.v.) injection of β-amyloid$_{1-42}$ protein oligomers (AβO) in mice.
To this end, C57BL/6 mice were injected i.c.v. with AβO and starting from 1 hour after the surgery, animals were treated daily by intraperitoneal injection with PQM130 (0.5–1 mg/kg) or donepezil (1 mg/kg) for 10 days. At the end of the treatment, the behavioral assessment was performed before the sacrifice.
Our results show that the treatment with PQM130 after the i.c.v. injection of AβO reduced oxidative damage and neuroinflammation and induced cell survival and protein synthesis through the modulation of glycogen synthase kinase 3β (GSK3β) and extracellular signal–regulated kinases (ERK1/2). Moreover, PQM130 increased brain plasticity and protected mice against the decline in spatial cognition.
In conclusion, the present study highlighted that PQM130 is a potent multi-functional agent against AD and could act as a promising neuroprotective compound.

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Identification of a thyroid hormone derivative as a pleiotropic agent for the treatment of Alzheimer's disease

Massimiliano Runfola[1], Michele Perni[2], Andrea Bacci[1], Serena Mero[3], Beatrice Polini[4], Maria Marchese[3], Grazia Chiellini[4], Daniela Giuliani[5], Sheraz Gul[6], Filippo Santorelli[3], Michele Vendruscolo[2] and Simona Rapposelli[1]

[1] *Department of Pharmacy, University of Pisa, Italy*
[2] *Chemistry of Health, University of Cambridge, UK*
[3] *Molecular Medicine, IRCSS Stella Maris, Italy*
[4] *Department of Pathology, University of Pisa, Italy*
[5] *Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Italy*
[6] *Drug Discovery Research ScreeningPort, Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Germany*

Alzheimer's disease (AD) is the most studied neurodegenerative disease and the leading cause of dementia. To date, AD represent the 6th most common cause of death worldwide, affecting over 45 million people, a figure predicted to double by 2030. The identification of effective pharmacological tools for AD represents one of the main medical challenges of our century. A critical issue in this quest for AD therapies is represented by its highly complex and poorly diagnosable pathogenesis. From protein homeostasis collapse to impairment of gut-microbiota axis, there is a tangled network of dysfunctional pathways cooperating to aggravate the AD phenotype. Increasing evidence shows that polypharmacology could be an intriguing route towards this end. Identifying DMT polypharmacological small molecules is, however, a complex and high-risk process, which requires a good starting point for drug design supported by a multidisciplinary network of *in vitro* and *in vivo* approaches. Towards this goal, we identified a thyroid hormone derivative, called SG2, with pleiotropic activity and a safe toxicological profile. SG shares a pleiotropic activity with its endogenous parent compound, including autophagic flux promotion, neuroprotection, and metabolic reprogramming. As we describe here, SG2 acts in a pleiotropic manner to induce recovery in a *C. elegans* model of AD based on the overexpression of Aβ42 and improves learning abilities in the 5XFAD mouse model of AD. Further, in vitro ADME-Tox profiling and toxicological studies in zebrafish confirmed the low toxicity of this compound, which could represent a starting point for drug development.

Effect of resveratrol-enriched nanospheres and resveratrol derivatives on a neuroprotective pathway involving neuroglobin accumulation

Emiliano Montalesi, Patrizio Cracco, Maria Marino

Department of Science, Roma Tre University, Viale Guglielmo Marconi 446, I-00146 Rome, Italy

The medicinal benefits of dietary polyphenols has been proved for several diseases including neurodegenerative diseases. Despite these data widespread the polyphenol consumption as dietary supplements, there is limited evidence of polyphenol therapeutic benefits as well as scarce attention has been reserved on their possible adverse side-effects. Indeed, the extensive metabolism to which polyphenols are exposed to into human body leads to their scarce concentration and persistence in blood paralleled by a plethoric number of metabolites that could possibly interfere with polyphenol protective action. Here, the neuroprotective effect of nanosphere-enriched and chemical modification of polyphenols has been evaluated on the accumulation of neuroglobin (NGB), which is at the root of estrogen/Estrogen Receptor β-induced protective effects against neuronal stress. The induction of NGB accumulation is a promising therapeutic strategy against neurodegeneration, although it could result in harmful effects, due the protective action NGB exerts on cancer cells. On this premise, a library of polyphenolic compounds has been screened to identify cell specific NGB modulators. Resveratrol has been selected as the most effective one, promoting NGB accumulation in neuroblastoma cells, while having a complete opposite action on breast cancer cells. Data obtained sustain that resveratrol conjugation with nanocarriers is more efficient than the compound alone or its chemical modification in modulating NGB accumulation maintaining the neuroprotective role of this globin against oxidative stress. This strategy could overcome the limits that the poor bioavailability imposes on the translation of polyphenols in therapeutical agents.

LRRK-2 inhibition by PF-06447475 treatment reduces neuronal damage and immune response after spinal cord trauma

Alessia Filippone[1], Deborah Mannino[1], Rossella Basilotta[1], Giovanna Casili[1], Irene Paterniti[1], Salvatore Cuzzocrea[1], Emanuela Esposito[1].

[1] *Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy*

Spinal Cord Injury (SCI) is a devastating event followed by neurodegeneration, activation of the inflammatory cascade and immune system. Dysregulated or non-resolving inflammatory processes can affect neuronal homeostasis and drive immune cells stimulation. The leucine-rich-repeat kinase 2 (LRRK2) is a gene associated with the progression of Parkinson's disease (PD), and its kinase activity was found to be upregulated after instigated inflammation of the Central Nervous System (CNS) and immune system activation. Here, we aimed to investigate the efficacy of PF-06447475, an LRRK2 inhibitor, by counteracting pathological consequences of spinal cord trauma.

The in vivo model of SCI was induced by extradural compression of the spinal cord at T6-T8 levels, then mice were treated with PF-06447475 (2.5-5 and 10 mg/kg o.s) 1 and 6 hrs after SCI. we found that PF-06447475 treatments at the higher doses (5 and 10 mg/kg) showed great abilities to reduce the degree of spinal cord tissue injury, glycogen accumulation, and demyelination of neurons associated with trauma. In addition, cytokines expression levels including interleukins (IL-1, IL-6, IL-10 and 12), interferon γ (IFN-γ) and tumor necrosis factor-α (TNF-α secreted and released by immune cells after trauma were decreased by LRRK-2 inhibitor treatments. Moreover, the accumulation of CD4+ and CD8+ cells throughout the spinal cord lesion site of SCI mice was reduced by PF-06447475 oral administration at the dose of 10 mg/kg.

Ferritin Nanocages loaded with Bisdimethylcurcumin as a new anti-inflammatory agent against Alzheimer's disease

Carlo Morasso[1], Stella Gagliardi[2], Veronica Tinelli[3], Marta Truffi[1], Maria Garofalo[2,4], Alfredo Costa[5,6], Giulia Perini[6], Matteo Cotta Ramusino[6], Davide Prosperi[3], Cristina Cereda[2], Fabio Corsi[1]

[1] *Nanomedicine and Molecular Imaging Lab, Istituti Clinici Scientifici Maugeri IRCCS Spa SB, Pavia Italy*
[2] *Genomic and post-Genomic Unit, IRCCS Mondino Foundation, Pavia, Italy*
[3] *Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milano, Italy*
[4] *Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia, Italy*
[5] *Department of Brain and Behavioral Sciences, University of Pavia, 27100 Pavia, Italy*
[6] *Unit of Behavioral Neurology, IRCCS Mondino Foundation, 27100 Pavia, Italy*

Bisdemethylcurcumin (BDC) might be useful as an inflammation inhibitor in AD patients. However, its clinical use is hampered by the fact that BDC is almost insoluble in water, poorly absorbed by the organism, and rapidly degraded. Therefore, we developed a new nanoformulation of BDC based on H-Ferritin nanocages (HFn) to solve this issue.
In this work, we tested the solubility and stability of the BDC-HFn and its ability to transverse the blood-brain barrier (BBB). Besides, we tested the effect BDC-HFn on PBMCs from AD and controls to evaluate the transcriptomic profile by RNA-seq.
The results obtained show that by using HFn it is possible to drastically improve the solubility of BDC and extend the stability for more than 24 hours. The BDC-HFn can bind endothelial cells from the cerebral cortex and cross through a BBB in vitro model with higher efficiency than the free drug. Transcriptomic analysis showed that a total of 630 Differentially Expressed RNAs (DEG), 350 up and 280 down-regulated between AD and controls untreated. The comparison between AD patients before and after BDC-HFn treatment showed a major number of DEG (2517). A further pathway analysis explicated that chemokine and macrophage activation are different between AD patients and controls and after BDC-HFn treatment.
In conclusion, our data showed how Hfn-BDC could improve the pharmacokinetic properties of the drug and that significant differences are present in the gene expression between the same patients before and after Hfn-BDC treatment in genes associated with inflammation.

DIETARY SUPPLEMENTS, NUTRACEUTICALS, AND FUNCTIONAL FOODS IN NEUROPROTECTION

Impact of a multinutrient in mild cognitive impairment due to Alzheimer's disease - LipiDiDiet

T. Hartmann, A. Solomon, P.J. Visser, A.M.J. van Hees[8], S.B. Hendrix, K. Blennow, M. Kivipelto, H. Soininen, on behalf of the LipiDiDiet clinical study group.

Diet is one of the major and most accessible risk factors for AD. It is believed that a change towards a healthy dietary pattern, if applied well before onset of overt dementia, will slow cognitive decline and possibly reduce the dementia risk.
For the last two decades the LipiDiDiet consortium has been investigating the role of nutrients and their synergism action on key AD pathological features. Based on preclinical results obtained, 11 nutrients were selected which, when applied in this specific combination, gave the most promising results in APP/PS transgenic AD-mouse models. These nutrients are the omega-3 fatty acids DHA and EPA, phospholipids, vitamins B6, B12, folic acid, C and E, as well as choline, selenium, and UMP, collectively this combination is now known as Fortasyn Connect (Souvenaid). Notably, when applied as composite formulation, synergistic effects are observed.
We evaluated Fortasyn Connect extensively in preclinical studies, and eventually tested it in a 6-year clinical trial with MCI-AD / prodromal AD participants with biomarker confirmed presence of AD pathology.
The currently available results cover the first 3 years of treatment. Significant slowing of the cognitive and functional decline, as well as reduced brain pathology was observed in the multinutrient treated participants compared to the placebo control participants. Notably, benefit increased with early and long-term intervention. We will present the recent trial results and discuss the implications and limitations.
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PALMITOYLETHANOLAMIDE, RELATED ALIAMIDES AND ANTIOXIDANT FORMULATIONS IN DIET: HEALTH AND WELLBEING

Rosalia Crupi[1], Enrico Gugliandolo[1], Salvatore Cuzzocrea[2]

[1] *Department of Veterinary Science, University of Messina, 8168 Messina, Italy;*
[2] *Department of Chemical Biological Pharmaceutical and Environmental Sciences, University of Messina, 98168 Messina, Italy*

Diet affect every cellular process and this represents the foundation of dietary management to a variety of several humans and animal disorders. Compounds that are present in nature, assimilable through diets, can therefore play an important role in maintaining the well-being of each individual. Recently great attention is being paid to a family of naturally occurring lipid amides acting through the so-called autacoid local injury antagonism, i.e., the ALIA mechanism. The parent molecule of ALIAmides, palmitoyl ethanolamide (PEA), has being known since the 1950s as a nutritional factor with protective properties. Since then, PEA has been isolated from a variety of plant and animal food sources and its proresolving function in the mammalian body has been increasingly investigated. Evidence indicates that PEA is an important anti-inflammatory, analgesic, and neuroprotective mediator acting on several molecular targets in both central and in peripheral organs and systems. The multitarget and highly redundant mechanisms through which PEA exerts prohomeostatic functions fully breaks with the classical pharmacology view of “one drug, one target, one disease”, opening a new era in the management of health, i.e., an according-to-nature biomodulation of body responses to different stimuli and injury. Moreover, the possibility of using PEA in association with other natural antioxidant molecules, such as the flavonoids, (Polydatin, Luteolin, Quercetin, Silyrmarin), demonstrates that PEA is able to act effectively not only individually, but also and above all in synergy with other molecules.

NEUROPROTECTIVE PROPERTIES OF EXTRACTS FROM *G. FRONDOSA* AND *H. ERINACEUS* IN MODELS OF NEURODEGENERATION

Farida Tripodi[1], L. Goppa[2], Manuela Leri[3], Monica Bucciantini[3], Elena Savino[2], **Paola Coccetti**[1]

[1] *Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy*
[2] *Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy*
[3] *Department of Experimental and Clinical Biomedical Sciences, University of Firenze, Firenze, Italy*

Nutrients and their metabolites control energy balance, enzymatic activities and genome stability throughout the lifecycle. Several results have suggested new roles of key nutrients in the protection of age-related disorders. Thus, there is an increasing interest in nutrition as a way both to prevent diseases and to reach healthy aging.
Medicinal mushrooms are commonly used in the prevention of many age-associated neurological dysfunctions, including Parkinson's disease (PD). Among the best performing edible mushrooms, *Grifola frondosa* and *Hericium erinaceus* are responsible for many health beneficial effects, including the improvement of cognitive functions (Fan et al. 2019, Rai et al., 2021).
Several studies underline the relevant role of cellular models for a better understanding of the molecular regulation of human pathologies. As such, budding yeast *Saccharomyces cerevisiae* has been extensively employed as a model of PD to dissect the molecular mechanisms of αSynuclein (αSyn) toxicity. Indeed, heterologous expression of human αSyn in wild type yeast cells results in both severe growth defects and in the accumulation of intracellular inclusions of αSyn (Tenreiro et al 2017).
Here, we show that acqueous extracts from dried sporophores of *H. erinaceus* and *G. frondosa* extend yeast lifespan in a Ras-dependent manner. Anti-aging and neuroprotective effects are observed also in cells expressing human α-Syn, with a reduction of ROS levels and protein aggregation. Overall, our findings are consistent with an inhibitory effect of these mushrooms on the formation of amyloid fibrils, suggesting important implications for treatment of age-related neurodegenerative diseases.

EVOO polyphenols effects on neuroinflammation associated with Alzheimer's disease

Manuela Leri[1], Marzia Vasarri[1], Federica Carnemolla[1], Donatella Degl'Innocenti[1], Monica Bucciantini[1]

1 Dipartimento di Scienze Biomediche Sperimentali e Cliniche "Mario Serio", Università degli Studi di Firenze

In Alzheimer's disease (AD), microglia, brain resident immune cells, become chronically inflammatory and neurotoxic. In the last years, neuroinflammation has attracted particular interest and genetic variants of molecules associated with ''microgliopathies'', including the triggering receptor expressed on myeloid cells-2 (TREM2), result in increased risk of developing AD and cognitive decline. TREM2 is expressed in brain microglia and modulates microglial functions in response to key AD pathology. Both oxidative stress and neuroinflammation precede over symptomology of neurodegenerative disorders and each promotes neurotoxic environments associated with neurodegeneration. In this context, natural polyphenols are considered as an alternative treatment mode for the prevention of neurodegenerative disorders due to their ability to limit activation of oxidative pathways and inflammation. The aim is to develop an unprecedented appreciation of the extra virgin olive oil (EVOO) polyphenols, oleuropein aglycone (OleA) and its main metabolite hydroxythyrosol (HT), as functional food against neuroinflammation and neuronal impairment. We performed a set of in vitro assays using neuronal and microglial cells model providing an integrated knowledge of the biochemical and cellular modifications inducing in AD and the mechanisms of protection by polyphenols. Moreover, we will improve the knowledge of basic aspects of AD and neuroinflammation, with particular relevance on TREM2 involvement.

Key mechanisms and potential implications of *Hericium erinaceus* in NLRP3 inflammasome activation by reactive oxygen species during Alzheimer's Disease

Rosalba Siracusa[1], Marika Cordaro[2], Roberta Fusco[1], Daniela Impellizzeri[1], Ramona D'Amico[1], Salvatore Cuzzocrea[1], Vittorio Calabrese[3], Rosanna Di Paola[1]

[1] *Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University of Messina, 98166 Messina, Italy*
[2] *Department of Biomedical, Dental and Morphological and Functional Imaging, University of Messina, 98125 Messina, Italy*
[3] *Department of Biomedical and Biotechnological Sciences, University of Catania, 95124 Catania, Italy*

Alzheimer's disease (AD) is the principal cause of dementia, and its incidence increases with age. Altered antioxidant systems and inflammation have an important role in the etiology of neurodegenerative disorders. In this study, we evaluated the effects of *Hericium erinaceus*, a nutritional mushroom with important antioxidant effects, in a rat model of AD. Animals were injected with 70 mg/Kg of AlCl3 daily for 6 weeks, and *Hericium erinaceus* was administered daily by gavage. Before the experiment's end date, behavioral test training was performed. At the end of the study, behavioral changes were assessed, and the animals were euthanized. Brain tissues were harvested for further analysis. AlCl3 mainly accumulates in the hippocampus, the principal region of the brain involved in memory functions and learning. *Hericium erinaceus* administration reduced behavioral changes and hippocampal neuronal degeneration. Additionally, it reduced phosphorylated Tau levels, aberrant APP overexpression, and β-amyloid accumulation. Moreover, *Hericium erinaceus* decreased the pro-oxidative and pro-inflammatory hippocampal alterations induced by AD. In particular, it reduced the activation of the NLRP3 inflammasome components, usually activated by increased oxidative stress during AD. Collectively, our results showed that *Hericium erinaceus* has protective effects on behavioral alteration and histological modification associated with AD due to the modulation of the oxidative and inflammatory pathways, as well as regulating cellular brain stress.

DIETARY SUPPLEMENTS, NUTRACEUTICALS, AND FUNCTIONAL FOODS IN NEUROPROTECTION

Bacopa monnieri and its Neuroprotective potential

Marcella Reale[1], Federica Serra[2], Lisa Aielli[1], Erica Costantini[3],

[1] *Department of Innovative Technologies in Medicine and Dentistry, University "G.d'Annunzio" Chieti-Pescara, Via dei Vestini, 66100 Chieti, Italy.*
[2] *Department of Pharmacy, University "G.d'Annunzio" Chieti-Pescara, Via dei Vestini, 66100 Chieti, Italy.*
[3] *Department of Medicine and Science of Aging, University "G. d'Annunzio" Chieti-Pescara, Via dei Vestini, 66100 Chieti, Italy.*

Bacopa monnieri (BME) is an herb that is used in the Ayurvedic medicine tradition for its anti-inflammatory activity. The human neuroblastoma cell line SH-SY5Y presents a neuronal structure like axons, dendrites, and perikaryon and are widely used as a cell model to study the mechanisms of neural death, neurotoxicity.
In this study, we evaluate the neuroprotective, anti-inflammatory and antioxidant properties of BME in *in vitro* model of neuroinflammation.
In SH-SY5Y cells the MTT assay, which evaluates mitochondrial activity through mitochondrial respiration, and LDH assay, which evaluates the enzymatic release, showed that cytotoxicity induced by 50 nM of Okadaic Acid (OA), was above 75%. The pretreatment of SH-SY5Y with 10 µg/ml of BME for 1 h, restored the viability to the extent of control cells ($P < 0.001$ OA+BME vs OA treated cells).
The *in vitro* model of traumatic brain injury, used to evaluate the potential neuroprotective ability of BME, showed a weakly reduction of stretch injury-induced iNOS expression and NO levels ($p=0.034$) as well as a significantly reduction of MMP-9 ($p<0.001$) in the presence of BME, accordingly with wound width reduction and with a significantly inhibition of IL-1β, IL-6 and COX-2 gene expression.
Thus, our results indicate that BME might become a promising candidate in the protection against neuroinflammation and neurotoxicity in the treatment of senile dementia progression, ageing complications as well as HIV-associated dementia. Additional studies will be needed to evaluate the potential use of BME in SARS-CoV-2-associated long-term neurological complications.

Perinatal supplementation with S-adenosylmethionine mitigates AD-like symptoms in adult TgCRND8 AD mice through PSEN1 CpG and non-CpG promoter methylation.

Tiziana Raia[1], Federica Armeli[2], Rosaria A. Cavallaro[3], Rita Businaro[2], Marco Lucarelli[1,4], **Andrea Fuso**[1]

[1]Dept. of Experimental Medicine, [2]Dept. of Medico-Surgical Sciences and Biotechnologies, [3]Dept. of Surgery "P. Valdoni", [4]Pasteur Institute, Cenci Bolognetti Foundation, Sapienza University of Rome, 00161 Rome, Italy

DNA methylation, which is one of the main epigenetic modifications modulating gene expression, is regulated by the metabolic pathway known as "one-carbon metabolism" in which S-adenosylmethionine (SAM) is produced. SAM is the main methyl-donor in the transmethylation reactions, that transfer methyl groups to different substrates, including DNA. We previously demonstrated that promoter hypomethylation of PSEN1, a gene involved in the amyloidogenic pathway in Alzheimer's Disease (AD), boosts the AD-like phenotype in transgenic TgCRND8 mice. On the contrary, supplementation with S-adenosylmethionine mitigates the pathological phenotype through PSEN1 promoter methylation, PSEN1 repression and consequent reduction of the amyloid burden.
Many evidences suggest that epigenetic signatures acquired during the first stage of life, or even in utero, can be revealed later on, driving the drift from normal to diseased aging.
We therefore studied the effect of post-weaning vs. perinatal SAM supplementation in TgCRND8 mice, respect to the AD-like phenotype and, specifically to PSEN1 modulation. We found that short term (from mother pregnancy to pups weaning) SAM perinatal supplementation is more effective than post-weaning chronic (3 months) supplementation in silencing PSEN1 expression via DNA methylation in adult mice. The effect on amyloid plaques reduction is comparable to that obtained by chronic SAM supplementation post-weaning.
These results point out the importance of methyl-donors availability during the early life as protective mechanisms towards neurodegeneration.

Eye neurodegeneration in hyperglycemic Drosophila melanogaster.

Elisabetta Catalani, Davide Cervia

Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), Università degli Studi della Tuscia, Viterbo, Italy

Nutraceuticals act on multiple intracellular signals and are considered as positive neuroprotectants of retinal cells. Hyperglycemia promotes microvascular complications, neurodegeneration, and angiogenesis, all of which contribute to retinopathy, including diabetic retinopathy (DR). There is evidence that in DR, early abnormalities in the neuroretina, triggered by hyperglycemia, could promote vascular impairments as a subsequent event. Adult wild-type *Drosophila melanogaster* (Dm) flies fed with high-sucrose diet exhibit typical signs of the initial stage of diabetes. Hyperglycemic flies showed a decrement in the responsiveness to light and neurodegeneration of photosensitive components. Furthermore, significant levels of cleaved caspase 3 and accumulated autophagosomes were detected in the internal retina network as well as nitrotyrosine, indicating apoptotic features, autophagy turnover defects and peroxynitrite formation. Therefore, this model could facilitate the exploration and treatments of the early degenerative features affecting the retina at both functional and molecular/cellular level, integrating more traditional vertebrate tools. There is a general agreement that nutrients could offer a new line of protection against DR. In this respect, we demonstrated that food supplementation with the natural antioxidant Lisosan G positively affects the visual system at structural/functional levels, decreased apoptosis, reactivated protective autophagy, and reduced the levels of brain ROS and retina peroxynitrite. This study demonstrated that the continuous supplementation with the alimentary integrator Lisosan G exerts a robust and multifaceted antioxidant effect on retinal neurons, thus providing efficacious neuroprotection of hyperglycemic eye.

Antidepressant activity of glucoraphanin in beta amyloid-induced depressive like behavior

Paolo Tucci[1], Maria Grazia Morgese[1], Stefania Schiavone[1], Maria Bove[1], Luigia Trabace[1]

Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

A large amount of glucoraphanin (GR) have been found in *Brassica oleracea*. Together with its anticancer effects in animal and cellular models, this compound has also shown beneficial effects in other chronic diseases and in central nervous system disorders. GR was reported to have antidepressant effects in a mice model of depression. Although the etiology of the depressive symptomatology is not fully understood, dysregulation of the central serotonergic and noradrenergic systems may be primarily involved. Moreover, a number of studies associated depressive state and inflammation, involving the kynurenine (KYN) metabolism pathway. Moreover, some form of depressions seems to be linked to the increase of soluble beta amyloid (sAβ) levels.
Hence, the aim of this work was to investigate the effect of GR in naïve and in sAβ-treated rats by using the forced swimming test, a widely used test to assess the capacity of antidepressant agents to switch passive behaviors in active forms of coping. We also evaluated whether serotonergic and noradrenergic neurotransmissions were affected by GR treatments, as well as the possible involvement of KYN and tryptophan alterations in the prefrontal cortex.

Neuroprotective Effects of Quercetin against Aflatoxin B1-Intoxicated Mice

Enrico Gugliandolo[1], Rosalia Crupi [1], Salvatore Cuzzocrea[2]

[1] *Department of Veterinary Science, University of Messina, Polo SS Annunziata, 98168 Messina, Italy*
[2] *Department of CHIBIOFARAM, University of Messina, Polo Papardo, 98166 Messina, Italy*

Aflatoxin B1 (AFB1) is a mycotoxin commonly present in feed, characterized by several toxic effects. AFB1 seems to have a neurotoxical effect that leads to memory impairment behavior. AFB1 toxicity involves the induction of the oxidative stress pathway, rising lipid peroxidation, and it decreases antioxidant enzyme levels. Hence, in our research, we wanted to evaluate the potential protective effects of quercetin 30 mg/kg in AFB1-mediated toxicity in the brain and the ameliorative effect on behavioral alterations. Oral supplementation with quercetin increased glutathione peroxidase (GSH) levels, superoxidedismutase (SOD) activity and catalase (CAT) in the brain, and it reduced lipid peroxidation in AFB1-treated mice. This antioxidant effect of quercetin in the brains of AFB1-intoxicated mice is reflected in better cognitive and spatial memory capacity, as well as a better profile of anxiety and lethargy disorders. In conclusion, our study suggests that quercetin exerts a preventive role against oxidative stress by promoting antioxidative defense systems and limiting lipid peroxidation.

* *presenter*

Olive oil polyphenols protect the brain from chronic low-grade inflammation

Daniel Di Risola[1*], Daniele Antinori[2], Matthew G. Pontifex[3], Gwenaelle Le Gall[3], Andrea Fuso[2], Anna Maggiore[1], Roberto Mattioli[1], Michael Caloisi[1], Maria d'Erme[1], Antonio Francioso[1], Michael Müller[3], Luciana Mosca[1§] and David Vauzour[3§]

[1] *Department of Biochemical Sciences A. Rossi Fanelli, Sapienza University, Rome, Italy*
[2] *Department of Experimental Medicine, Sapienza University, Rome, Italy*
[3] *Norwich Medical School, University of East Anglia, Norwich, UK*
[§] *These authors share senior authorship:* luciana.mosca@uniroma1.it ; D.Vauzour@uea.ac.uk

The unsaponifiable fraction of extra virgin olive oil (EVOO) contains polyphenols showing antioxidant and anti-inflammatory activity. Epidemiological evidence indicates that regular intake of olive oil may be beneficial to dampen aging-associated cognitive decline.
We tested whether EVOO polyphenols protect from cognitive deficit in an animal model of low-grade chronic inflammation (i.p. injection of 0.5 mg/Kg bw LPS once a week for 8 weeks). We used olive oil extract (OOE, from Coratina oil) dissolved in drinking water (10mg/Kg bw/day). Male C57Bl/6J mice were were divided into 4 groups: 1) Saline injected mice who drank water (Sham/Water); 2) Saline injected mice who drank OOE water (Sham/OOE); 3) LPS injected mice who drank water (LPS/Water); 4) LPS injected mice who drank OOE water (LPS/OOE). After 8 weeks mice were assessed for cognitive behaviour by the Novel Object Recognition test before sacrifice and organs collected. Gut microbiota diversity and metabolism were assessed by 16S rRNA sequencing and ^{1}H NMR metabolomics respectively. Proinflammatory cytokines, NLRP3, iNOS, carbonylated proteins and lipooxidation were assessed via Western blot, ELISA and qRT-PCR. Data indicate that OOE protects cognitive functions from LPS damage and induces a shift in intestinal bacteria by increasing Desulfobacteria and reducing Actinobacteria, both in Sham/OOE and in LPS/OOE groups. No systemic inflammation or oxidative stress were evidenced in LPS treated mice, whereas the combined treatment (LPS/OOE) decreased proinflammatory markers compared to OOE group. Our findings demonstrate that OOE supplementation may have triggered an hormetic response through the induction of a mild stress which in turn elicited protective responses in the organism.

Dietary Polyphenols as Restoring of Protective Pathways against Neurodegeneration

Patrizio Cracco, Maria Marino

Department of Science, University Roma Tre, Rome, Italy.

Although the incidence of Neurodegenerative Diseases (NDs) has been increasing in these last decades, still there are only palliative remedies to cure these pathologies. We hypothesized that it is possible to delay and/or prevent the onset of NDs by restoring one of the physiological endogenous neuroprotective pathways. Very promising for this role is Neuroglobin (NGB), an inducible globin which accumulation protects brain against different stressors. Among the various NGB inducers, we identified the activation of 17β-estradiol (E2)/estrogen receptorβ (ERβ) pathway, which lead to the NGB accumulation into mitochondria, where the globin increases cell resilience to the apoptosis induced by various stressors. Regrettably, the E2/ERβ/NGB axis is one of the pathways impaired during neurodegeneration. Here, we verify if natural (i.e., resveratrol and naringenin) or synthetic (i.e., diarylpropionitrile) molecules, which are ERβ ligands, could mimic E2 reactivating NGB accumulation and its protective effects. Neuroblastoma cell lines (SK-N-BE, SHSY5Y), mutated striatal cells and neuronal-derived cells treated with 3-NP, respectively genetic and chemical model of Huntington Disease were used as experimental model. Our results demonstrate that both natural and synthetic molecules increase NGB levels even in the genetic models of neurodegeneration, via ERβ, by activating specific pathways that preserve cells against various stressors and protect these cells from the apoptosis.

Exploring the neuroprotective potential of the natural coumarin esculetin: *in vivo* and *in vitro* evidences

Letizia Pruccoli [1] and Andrea Tarozzi [1]

[1] *Department for Life Quality Studies, University of Bologna, 47921 Rimini, Italy*

Coumarins are an important group of natural compounds widely distributed in the natural kingdom with strong pharmacological activities, low toxicity and high bioavailability [1]. Interestingly, some natural coumarins showed the ability to inhibit monoamine oxidase, acetylcholinesterase and butyrylcholinesterase with great potential as neuroprotective agents [2]. In this context, the coumarin esculetin exhibited several *in vivo* and *in vitro* neuroprotective effects by acting on multiple targets. In particular, esculetin showed the ability to cross the blood–brain barrier and prevent the dopaminergic neuronal death in a mouse model of Parkinson's disease [3]. Further, esculetin prevented and counteracted the reactive oxygen species formation in neuronal SH-SY5Y cells by promoting the nuclear translocation of Nrf2 and the subsequent increase of glutathione. In similar experimental conditions, esculetin protected the SH-SY5Y cells from the oxidative stress and neuronal death evoked by oligomers of amyloid beta peptide through the activation of Akt and Erk1/2 signaling pathways [4]. Esculetin also demonstrated to partially inhibit the progression of mutant huntingtin (mHTT) aggregation and reduce the neuronal death through its ability to counteract the oxidative stress and mitochondria impairment elicited by mHTT in an inducible PC12 model of Huntington's disease (HD). The ability of esculetin to counteract the neuronal death was then confirmed in a transgenic *Drosophila* model of HD [5]. In conclusion, these results encourage further research to better explore the neuroprotective potential of the natural coumarin esculetin as drug or functional food for preventing or treating neurodegenerative diseases.

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PHYSICAL ACTIVITY AND EXERCISE IN NEUROPROTECTION

Rehabilitation of peripheral neuropathy and synergic control of posture: NEUPER study

Miryam Mazzucchelli[1,2], Paola Alberti[3,4], Cristiano Alessandro[5], Cecilia Perin[2,5], Daniele Piscitelli[5], Guido Cavaletti[3,4]

[1] *PhD program in Neuroscience, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy*
[2] *Istituti Clinici Zucchi, San Donato Group, Carate Brianza, Italy*
[3] *Experimental Neurology Unit, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy.*
[4] *NeuroMI (Milan Center for Neuroscience), Milan, Italy*
[5] *School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy*

Peripheral perception disorders may impair movement control and cause abnormal muscular activation. Rehabilitation intervention may improve postural capacity. Synergy Index (SI) and Anticipatory Synergy Adjustments (ASAs) are new measurement of postural stability.
NEUPER study aims to characterize multi-muscle synergy organization in relation to postural stability in individuals affected by peripheral neuropathy (PN) and to assess the effectiveness of a rehabilitation program driven by SI and ASA findings on clinical outcomes. Participants will be assessed at baseline, after a 20 session 2-3/week rehabilitation program and at 3 months after treatment. Outcome measures are: Total Neuropathy Score–clinical version (TNSc), nerve conduction studies, Short Form Health Survey 36 (SF-36), Functional Independence Measure (FIM), SixMinuteWalkingTest (6MWT), MiniBalanceEvaluation SystemTest (MiniBESTest), TimedUpandGotest (TUG) and postural evaluation recording center of pressure displacement via a force platform and surface electromyography. Rehabilitation treatment aim to a relearning of disrupted strategies for orthostatism, transfer and ambulation and included neurodynamics techniques.
Three participants (mean age(ds):64.08(10.06)) suffering from PN due to chemotherapy treatment for breast cancer were recruited. Preliminary data at baseline showed that all subjects had a grade 2 PN according to TNSc. Physical and mental health summary scores of SF-36 were above the mean compared to sex ad age matched reference population, apart for one participant (only for mental health). FIM scores indicated a modified/complete functional independence. 6MWT showed a decreased aerobic capacity in all subjects. Two subjects resulted at risk of fall at MiniBESTest, while none according to TUG. SI and ASAs evaluation is being processed.

IMPACT OF PHYSICAL EXERCISE ON BRAIN STRUCTURE, COGNITIVE AND MOTOR PERFORMANCE IN THE ELDERLY

Mandy Bade[1], Anita Hökelmann[1], Jörn Kaufmann[1], Volkmar Leßmann[1], Patrick Müller[2], Kathrin Rehfeld[1], Notger Müller[1]

[1] *Otto von Guericke University Magdeburg, Germany*
[2] *German Center for Neurodegenerative Diseases in Magdeburg, Germany*

The growing number of elderly people in the population is accompanied by an increased prevalence of chronic diseases (e. g. dementia) which will confront the healthcare system with major challenges in the future. Therefore, appropriate interventions are needed to support healthy aging combined with a high quality of life.
In a longitudinal study with 20 healthy elderly people (M=72.65, SD=4.31 years) the influence of endurance and strength training as well as dance training on brain structure, BDNF, memory, balance ability and endurance performance was investigated. Measurements were performed before the start of the training phase, after 6 months, 18 months and 5 years. Structural MRI scans, blood analyses, the Verbal Learning and Memory Test, Limits of Stability Test (LoS) and Physical Working Capacity Test 130 (PWC 130) were conducted.
In both groups, we observed an increased volume in the left amygdala, a maintenance of BDNF concentration in blood plasma, an improvement in balance ability according to LoS in terms of reaction time, movement velocity, endpoint, maximum dislocation and direction control as well as a stabilization of the performance in PWC 130. Memory performance improved significantly in the dance group with regard to recognition performance.
The neuroplasticity effect was confirmed as a result of both training measures based on neurostructural, molecular, cognitive, coordinative and conditional adaptation phenomena in the adult organism. The study showed that a long-term physically active lifestyle leads to preservation of performance and thus to a higher quality of life in old age.

Neuro-exergaming for mild cognitive impairment (MCI): Nutrition and diversity status of pilot enrollees in the interactive Physical and Cognitive Exercise Study (iPACES)

Paul Arciero[1,3], Darlingtina Esiaka[2], IreLee Ferguson[3], David Merrill[4], Stella Panos[4], Cay Anderson-Hanley[3,5]

[1] *Health and Human Physiological Sciences, Skidmore College (NY)*
[2] *Robert Wood Johnson Medical School, Rutgers University (NJ)*
[3] *iPACES LLC (NY)*
[4] *Pacific Brain Health Center, Providence Neurosciences Institute (CA)*
[5] *Psychology & Neuroscience, Union College (NY)*

The increasing prevalence of Alzheimer's Disease and related dementias (ADRDs) is a worldwide concern, and thus efforts to stem cognitive decline include an array of interventions. A vast literature supports the efficacy of exercise for brain health, but most elders do not exercise sufficiently. We are conducting an RCT for mild cognitive impairment (MCI patient and caregiver) of an affordable in-home neuro-exergame: iPACES (the interactive Physical and Cognitive Exercise System); it utilizes a tablet-based game interconnected with an under-table pedaler tailored to target specific neuropsychological functions. Participants are enrolled remotely, in part due to the on-going COVID-19 pandemic, but also to increase accessibility across the USA. We examined the nutritional status of pilot enrollees to gauge the possibility of nutrition interventions, including improving food access and a prescriptive dietary approach (Protein Pacing). Initial participants (n = 10 from five households) completed baseline neuropsychological assessments via videoconference, and questionnaires on the tablet, including the Food Access Questionnaire and a 4-day diet log. Results indicate enrollees are mostly Caucasian, with high SES and have good access to quality food, as all reported they can drive to a full-service grocery. Only one participant didn't think they ate enough fruits and vegetables. Diet logs confirm quality food intake, with mostly sufficient fruits and vegetables across the sample, but room for improvement in the realm of protein intake. Implications include increasing sample diversity, while minding access issues should we pursue potential additional benefit to cognition of supplementing the core iPACES exercise with a nutrition intervention.

Sleep and Motor learning

Antonio Cicchella

Department for Life Quality Studies, University of Bologna (Italy)

Recent works on learning and sleep considered the possible effect of sleep on motor skill learning (Laureys, 2002). A large body of evidence, spanning a wide range of neuroscientific disciplines, now describes evidence of sleep-dependant learning in both humans and animals (Walker & Stickgold, 2004) already complemented by cellular and molecular models of sleepdependent plasticity. Growing evidence suggests that sleep plays an important role in the process of procedural learning and, most recently, sleep has been found to be implicated in the continued development of motor-skill learning following initial acquisition. Within the procedural memory domain, sleep in human has been shown to trigger significant overnight learning enhancements, whereby performance is selectively improved across sleeping intervals, while equivalent waking periods confer no such performance benefit (Walker, 2005). Demonstrations of overnight sleepdependent learning have now been reported across both sensory (Karni et al., 1994; Gaab et al., 2004) and motor skill memory domains (Fischer et al., 2002; Walker et al., 2003; Kuriyama et al., 2004). Several different factors are considered to be important in procedural memory formation. One of the critical elements is the amount of training or practice during task acquisition, which strongly influences both behavioural learning and functional brain changes. It is widely accepted that, following effective acquisition, a specific memory representation is formed, which can then undergo further modification during the process of consolidation. The other fundamental factor is the passage of time. Infact, practice is vital to the acquisition of new skills, but the brain does not stop processing the information when practice stops. After practice, changes take place that strengthen and modify the new skill. These changes, describes under the umbrella term ‘consolidation’, take two distinct forms: the enhancement of skills and the stabilization of memories. How time passes seems to be a major contributor to memory consolidation in the socalled ‘latent period’. This presentation reviews the state of the art of sleep and motor learning in humans.

POSTERS

Development of New Potential MAO-B/αp38-MAPK Dual Inhibitors to Target Neuroinflammation In Alzheimer's Disease

Claudia Albertini[1], Thales Kronenberger[2], Mark Kudolo[2], Francesca Massenzio[1], Sabrina Petralla[4], Fernanda Borges[3], Gert Fricker[4], Barbara Monti[1], Stefan Laufer[2], Maria Laura Bolognesi[1]

[1] *Department of Pharmacy and Biotechnology, University of Bologna, 40126, Bologna, Italy.*
[2] *Department of Pharmaceutical/Medicinal Chemistry, University Tübingen, 72076 Tübingen, Germany.*
[3] *CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007, Porto, Portugal*
[4] *IPMB - Pharmazeutische Technologie und Biopharmazie, Universität Heidelberg, D-69120 Heidelberg.*

Alzheimer's Disease (AD) is the major cause of dementia worldwide.[1] Unfortunately, there are currently no drugs available to prevent or treat AD. The complex and multifactorial nature of AD makes the classical paradigm "one-target-one-drug-one disease" ineffective. In this scenario, a promising strategy is represented by multi-target-directed ligand (MTDL) polypharmacological strategy.[2] MTDLs are single-molecules able to modulate simultaneously multiple pathological pathways, obtaining a synergic effect.[2] Since, neuroinflammation is an important contributor to AD pathogenesis and progression, a promising ongoing phase-2 clinical trial investigates neflamapimod (VX-745,) administration to AD patients.[3] Neflamapimod, is a selective αp38-MAPK inhibitor, it was initially developed as an anti-inflammatory agent for rheumatoid arthritis but it failed because of its central side effects. However, neflamapimod was recently repurposed for AD treatment. αp38 Mitogen-Activated Protein Kinase (αp38-MAPK) is deeply involved in neuroinflammation, promoting cytokine and chemokine production and microglia response.[3] Moreover, monoamine oxidase (MAO-B) is a target involved in several neurodegenerative diseases and it was found up-regulated in neuroinflammatory processes. Thus, aiming in modulating synergistically different neuroinflammatory pathways, we designed and synthesized new potential dual-inhibitors to inhibit αp38-MAPK and MAO-B. By following a ligand-based approach, we introduced the propargylamine fragment of rasagiline, a selective MAO-B inhibitor currently used to treat Parkinson's disease, on neflamapimod's scaffold. So far, docking studies predicted αp38-MAPK and MAO-B inhibition by the obtained compounds, and experimental studies confirmed their αp38-MAPK inhibitory activities. Further studies to evaluate MAO-B inhibition, blood-brain-barrier permeation, and immunomodulatory and neuroprotective properties of the tested compounds are currently ongoing.

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The melatonin effect on the F_1F_O-ATPase activity, the mitochondrial respiration and the permeability transition pore

Cristina Algieri[1], Veronica Turchi[1], Giampaolo Morciano[2,3], Fabiana Trombetti[1], Paolo Pinton[2,3], Salvatore Nesci[1]

[1] *Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra, 50, Ozzano Emilia, 40064 Bologna, Italy*
[2] *Maria Cecilia Hospital, GVM Care&Research, 48033, Cotignola, Italy*
[3] *Department of Medical Sciences, Section of Experimental Medicine, Laboratory for Technologies of Advanced Therapies (LTAT), University of Ferrara, 44121, Ferrara, Italy*

Melatonin is a polyhedral molecule with direct antiradical activity, indirect antioxidant (1), anti-inflammatory and the ability to improve mitochondrial function (2). On swine heart mitochondria, melatonin is tested on the hydrolytic activity of F_1F_O-ATPase activated with the natural cofactor Mg^{2+} or Ca^{2+}, the latter condition associated with the mitochondrial permeability transition pore (mPTP) formation (3). The data suggest that melatonin inhibits Ca^{2+}-activated F_1F_O-ATPase with an uncompetitive mechanism by interacting on the F_1 portion of the enzyme, while the inhibitory effect on Mg^{2+}-activated F_1F_O-ATPase is temperature-dependent and increases with decreasing temperature. Moreover, mitochondrial respiration is inhibited by melatonin at the first respiratory substrate site. The melatonin may prevent the electron transfer from complex I to downhill respiratory chain complexes, whereas the succinate-O_2 oxidoreductase activity is refractory to melatonin. However, the coupling between substrate oxidation (glutamate/malate or succinate) and ADP phosphorylation, evaluated as State 3/State 4 ratio, is unaffected by melatonin. Studies on mPTP show desensitization of its opening by melatonin, in agreement with the inhibition of Ca^{2+}-activated F_1F_O-ATPase. ROS production is inhibited when mitochondria are energized with pyruvate/malate or succinate. The data obtained allow us to clarify the molecular mechanism of action of melatonin at the mitochondrial level showing a beneficial effect as an inhibitor of mPTP and strengthening its role as an indirect antioxidant agent.

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miR-218 in neurodegeneration and tumor reversion

Daniele Antinori[1], Tiziana Raia[1], Marco Lucarelli[1,2], Mariano Bizzarri[1] and Andrea Fuso[1].

[1] *Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy.*
[2] *Pasteur Institute Cenci Bolognetti Foundation, Sapienza University of Rome, Rome, Italy.*

In recent times, total embryo extract or isolated embryo factors have being getting increasing attention due to their ability to revert pathological conditions, including tumor and neurodegenerative processes. We performed a PCR array to evaluate the ability of the 20-somite state zebrafish embryo extract in modulating the microRNAs (miRNAs) expression in tumor (MDA-MB-231 and in MCF-10A committed toward Epitelial-Mesenchymal Transition) and neurodegenerative (SK-N-BE) cell line models. Intriguingly, miRNA-218-5p was shown to be modulated by embryo extracts in these cell lines. Deregulation of miRNA-218-5p is known to be involved in tumor development and neurodegeneration, and its expression is mainly regulated by the methylation status of its promoter. Therefore, we studied the modulation of miRNA-218-5p expression in MDA-MB-231 and SK-N-BE in iper- and ipo-methylating condition, respectively induced by S-adenosylmethionine (SAM) supplementation and B-vitamin deficiency in the growth medium. miRNA-218-5p is significantly downregulated in iper-methylating condition, while in ipo-methylating condition no appreciable changes were observed. Since the miRNA-218-5p is trascribed by SLIT2 sequences, we studied CpG and non-CpG methylation profile of its promoter. The methylation profile study reveals that in both cell lines, the iper-methylating condition modifies the methylation pattern leading to an increase in the overall methylation status.
In conclusion, both embryo extracts and SAM can significantly dowregulate miRNA-218-5p, representing a possible mechanism through which it could exerts a reversion of neurodegeneration.

ANTI-INFLAMMATORY ACTIVITY OF BLUEBERRY EXTRACTS

Armeli F[1], Maggi E[1], Mengoni B[1], De Caris MG[2], Francioso A[3], Grieco M[3], Mosca L[3], Pinto A[2], D'Erme M[3], Mancini P[2], Businaro R[1]

[1] *Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome*
[2] *Department of Experimental Medicine, Sapienza University of Rome*
[3] *Department of Biochemical Sciences, Sapienza University of Rome*

Antioxidant and pharmacological properties in blueberry components are known.
In this study, we evaluated the effect of blueberry hydroalcoholic extract (BB) in cells of the monocyte-macrophage lineage stimulated with the lipopolysaccharide (LPS). Central nervous system innate immunity cells, microglia, release inflammatory mediators during acute insults, polarizing towards a pro-inflammatory phenotype. We analyzed the mRNA expression of pro-inflammatory cytokines (IL-1b, IL-6 and TNF-a) in BV2 cells treated with BB in the presence or absence of LPS and the results show a significant anti-inflammatory effect of BB. By immunofluorescence analysis we also analyzed the expression of the main microglial phenotypic markers and the results show a decrease of iNOS (M1 marker) and an increase of ARG-1 (M2 marker) in cells incubated with BB.
The morphological changes in microglia derive from a cytoskeletal reorganisation so the activity of GTPases regulating the migratory capacity and structure of the actin, Rho and Rac1 cytoskeleton was assessed. BB extract stopped the increase in Rac1 activity after stimulation with LPS.
Our results show that, during the inflammatory response, BB extract shifts the M1 polarization towards the M2 phenotype through an actin cytoskeletal rearrangement. Based on that, we might consider BB as a nutraceutical with anti-inflammatory activities.

Vitamin D attenuates neuronal aging: synergistic effects with environmental enrichment and nutraceuticals

Martina Balducci[1] and Andrea Tarozzi[1]

[1] *Department for Life Quality Studies, University of Bologna, 47921 Rimini, Italy*

Aging generally refers to a biological complex process accompanied by stress related toxic products, methylation of DNA and mitochondrial damage that leads to an accelerated cell death, an increase in inflammation and a decrement in neuronal plasticity. Moreover, telomere shortening and subsequent cell senescence lead to tissue aging and age-related diseases. Vitamin D prevents neuronal aging and its cognitive-related processes, protecting cells from oxidative-stress damage, regulating protein expression and stimulating neurotrophic factors. Furthermore, it is recent evidence that the action of Vitamin D is enhanced when combined with other factors, such as the environment or different nutraceuticals. The combination between Environmental Enrichment (EE), which attenuates the age-related cognitive decline, and Vitamin D (400 IU kg−1 daily) has been tested on male aged rats and compared to three other housing conditions (environmentally enriched (EE), socially enriched (SE), or standard condition (SC). The treatment with EE and Vitamin D showed the higher score in memory and learning, in the expression of mRNA levels of neurotrophic factors (NGF, TrkA, BDNF, Nrf2, and IGF-1) and a greater enhancement of hippocampal LTP and neuronal excitability, when compared to EE alone. Additionally, a nutraceutical approach has demonstrated that a novel natural formulation containing Centella asiatica extract, vitamin C, zinc and vitamin D3 enhances telomerase expression and telomerase activity in the brains of rats. These data suggest that a synergistic action among Vitamin D and different environmental factors improves the protection exerted by vitamin D in neuronal aging.

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NEWLY SYNTHETIZED A_{2A}AR SELECTIVE ANTAGONISTS MODULATES NEUROINFLAMMATION AND REPRESENTS A PROMISING STRATEGY IN PARKINSON'S DISEASE

Maria Cristina Barbalace [1], Michela Freschi [1], Catia Lambertucci [2], Silvana Hrelia [1], Cristina Angeloni [2]

[1] *Department for Life Quality Studies, Alma Mater Studiorum University of Bologna, Bologna, Italy*
[2] *School of Pharmacy, University of Camerino, Camerino (MC) Italy*

Parkinson's Disease (PD) is one of the most common neurodegenerative disorder worldwide and a health concern in ageing societies. Neuroinflammation, a key event in PD, is driven by astroglia and microglia which represent the brain resident immune cells. Dysregulation of these cells leads to abnormal release of inflammatory cytokines like tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), interferon-γ (IFN-γ) and the increase of oxygen reactive species (ROS) [1]. The activation of microglial cells can be mediated by adenosine through the interaction with A_{2A} adenosine receptors (A_{2A}AR). Indeed, a slower degeneration of nigrostriatal dopaminergic cells in PD can be achieved modulating purinergic receptors [2].

A_{2A}AR antagonist 8-ethoxy-9-ethyladenine (ANR94) showed to protect nigrostriatal neurons from neuroinflammation in an animal model of PD [3], so aim of this project was to test several A_{2A}AR antagonists (ANR94 analogues) with improved pharmacodynamic and pharmacokinetic properties, on BV-2 microglial cells activated with 100 ng/mL LPS.

Among the synthesized compounds thirteen of them with the highest affinity and/or selectivity for the A_{2A} subtype have been further characterized. Their anti-inflammatory activity was evaluated by measuring the release of NO in the culture medium by Griess reagent, while gene expression analyses of cytokines IL-1β and IL-10, and the pro-inflammatory enzymes iNOS, COX-2 and NLRP3 were performed by RT-PCR. Interestingly, among the newly synthetized compounds, that identified with the number 13 would seem to be the most promising in counteracting inflammatory damage suggesting its potential use as therapeutic agent to prevent/counteract PD. Animal studies are ongoing to investigate its in vivo activity.

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Kynurenic acid administration after hypoxia-ischemia on neonatal rats reduces oxidative stress.

Bratek E.[1], Ziembowicz P[1]., Godlewski J[2]., Salinska E[1].

[1] *Dept. Neurochem. Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland.*
[2] *Tumor Microenvironment Laboratory, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland.*

Cerebral hypoxia-ischemia (HI) at the time around birth is one of the leading causes of neonatal mortality and neurological disabilities. KYNA (kynurenic acid) is a potent endogenous antagonist of NMDA receptors, as well as a free radical scavenger inhibiting oxidative stress.
Aims: The present study was aimed to investigate the ability of KYNA to reduce HI evoked brain injury and oxidative stress in experimental HI on rats.
Methods: 7-day old rat pups were anesthetized and the left common carotid artery was isolated and cut between ligatures. 60 min later the pups were subjected to hypoxia (7.4% O_2) for 75 min. Animals were injected i.p. with different doses of KYNA 1 h or 6 h after HI. Brains were analyzed for brain damage, the level of radical oxygen species (ROS) and glutathione (GSH), the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured.
Results: The application of KYNA reduced the loss of tissue weight in the ischemic hemisphere decreased the damage of neuronal cells in the cortex and the CA1. KYNA reduced the ROS level in the ischemic hemisphere compared to the HI group. KYNA application decreased also the expression of SOD, catalase, and GPx activity in the ischemic hemisphere compared to HI. The neuroprotective effect was better expressed when KYNA was administered 1 h after H-I.
Conclusions: The results show the neuroprotective effects of KYNA applied in a short time after H-I. The results indicate that neuroprotection is connected with a reduction of oxidative stress.

Key role of A_1 and A_{2A} adenosine receptors in neuroprotection

Michela Buccioni, Diego Dal Ben, Beatrice Francucci, Catia Lambertucci, Gabriella Marucci, Andrea Spinaci, Rosaria Volpini

School of Pharmacy, Medicinal Chemistry Unit, University of Camerino, Via Madonna delle Carceri, 62032, Camerino, Italy

Neuroinflammation is a common aspect of numerous neurodegenerative diseases and age-associated cognitive impairment. The identification of pathways involved in this process and the use of pharmacological strategies targeted against molecules activated during the inflammatory state are attracting a lot of attention. The autacoid adenosine, endogenous ligand of four adenosine receptors (A_1AR, A_{2A}AR, A_{2B}AR, A_3AR), seems to modulate astrocytes and microglial cells that are activated during neuroinflammation by interaction with A_1 and A_{2A}ARs. Evidences suggest that A_1AR activation produces a neuroprotective effect and A_{2A}ARs block prevents neuroinflammation.
A recent published manuscript showed that neuroinflammation and cellular apoptosis, promoted by a cytokine (CK) cocktail, can be alleviated by the A_1 partial agonist 2'-dCCPA and the A_{2A} antagonist 8-chloro-9-ethyl-2-phenethoxyadenine [1]. The first was able to prevent the inflammatory state induced by CKs, while the second has both anti-inflammatory and antioxidant properties, preventing neuroinflammation in mixed glial cells.
Since glial cells have functions in support, nutrition and defense of central nervous system but no direct synaptic transmission, the aim of this work was to evaluate the effect of these compounds on neurons, the main responsible for nerve transmission.
Treatment of these cells with the compounds, in presence and absence of CKs, demonstrated to restore cell health after the aggression with CKs, corroborating the results obtained in mixed glial cells. To elucidate the pathways that could be enrolled after receptor activation, some intracellular molecules were checked by western blot. Results demonstrated the involvement of cytochrome C, inducible nitric oxide synthase and caspase-3.

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Characterization of herbal medicine as novel GSK-3β inhibitors endowed with multi-targets neuroprotective effects

Angela De Simone[1], Serena Montanari[2], Lara Davani[2], Eliska Emrichova[2], Vincenza Andrisano[2], Cristina Delerue-Matos[3] and Clara Grosso[3]

[1] *Department of Drug Science and Technology University of Turin, via P.Giuria 9, 10125 Torino, Italy*
[2] *Department for Life Quality Studies, Alma Mater Studiorum-University of Bologna, 47921 Rimini, Italy*
[3] *REQUIMTE/LAQV, Instituto Superior de Engenharia do Instituto Politécnico do Porto, Rua Dr.António Bernardino de Almeida, 431, 4249-015 Porto, Portugal*

Due to the different and relevant roles covered in many pathways involved in the development of neurodegenerative disorders, GSK-3β is considered an attractive target to be addressed for the treatment of such diseases like Alzheimer's Disease (AD)[1]. The most relevant GSK-3β inhibitors characterized so far, have a natural origin and belong to alkaloid or flavonoid classes[2]. However, the multi-targets directed ligand approach is considered more promising to discover new entities which can halt or delay neurodegeneration[3]. In order to discover new natural entities able to target GSK-3β and other hallmarks associated with AD, seven decoctions obtained from the species *Scutellaria baicalensis* Georgi, *Ginkgo biloba* L., *Hypericum perforatum* L., *Curcuma longa* L., *Lavandula angustifolia* Mill., *Trigonella foenum-graecum* L. and *Rosmarinus officinalis* L., have been characterized in terms of GSK-3β inhibition, as well as cholinesterase inhibition, and for their ability to scavenge four different free radicals (DPPH$^{\bullet}$, ABTS$^{\bullet+}$, $O_2^{\bullet-}$ and $^{\bullet}$NO). All extracts were found to moderately inhibit GSK-3β and able to scavenge free radicals. Moreover, they displayed weak to moderate acetylcholinesterase and butyrylcholinesterase inhibition. The inhibitory activity towards GSK-3β could be ascribed to the presence of several flavonoids, phenolic acids, curcuminoids, phenolic diterpenoids, one alkaloid and one naphthodianthrone, identified and determined by HPLC analyses in the seven decoctions[4]. The obtained results confirm natural products as an invaluable source of active molecules underlining the opportunity to find in plant extracts some pharmacological entities able to combat multifactorial disorder like AD.

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C. *elegans* models of neurodegeneration to investigate the effect of specific dietary components on protein misfolding and animal longevity

Margherita Romeo, Marten Beeg, Marco Gobbi, **Luisa Diomede**

Department of Molecular Biochemistry and Pharmacology, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy

Aging is associated with the gradual accumulation of protein aggregates that contribute to senescence and determine the onset of neurodegenerative diseases. A large proportion of this heterogeneous group of disorders is defined by the sporadic central deposition of amyloid β (Aβ) aggregates suggesting that non-genetic actors, such as lifestyle and environment, may play a role in their incidence.
Diet is one of the most changing aspects of lifestyle due to the different nutrients to which organisms are exposed. In recent years, great efforts have been spent trying to reveal whether nutritional factors can influence the molecular mechanisms underlying aging in both physiological and pathological conditions.
Using the nematode *C. elegans* as a simplified model of Aβ-induced proteotoxicity, we investigated the effect of specific dietary components on protein misfolding and animal longevity. This worm represents a unique tool for rapidly obtaining relevant information on physiological and pathological changes in aging and protein aggregation, enabling otherwise long-term and costly studies in vertebrates.
Studies conducted on transgenic strains of *C. elegans* expressing the human Aβ1-42 peptide have shown that natural compounds such as oleuropein (a polyphenolic compound in virgin olive oil), the green tea constituent epigallocatechin gallate and silybin (the main active constituent of silymarin), increase the lifespan of the worm and protect them from amyloid proteotoxicity.
These findings suggest that specific nutrient intake may result in a protective biological effect on aging mechanisms and provide proof of concept for future vertebrate studies.

Abscisic acid Supplementation and Alzheimer disease: Effects in mRNA levels of BDNF and IRS2 in the 3xTg mice model

Verónica Espinosa-Fernández[1], Aroa Mañas-Ojeda[2], Francisco Ros Berna1[2], Ernestina Castro-Salazar[3], Mar Herrero-Pacheco[3], Ana M Sanchez-Perez[1]

[1] *Neurobiotecnología , INAM, Universitat Jaume I*
[2] *Departamento de Medicina, Universitat Jaume I*
[3] *Departamento de Medicina, Pontificia Universidad Católica Madre y Maestra, Dominican Republic*

Neuroinflammation and brain insulin resistance are intimately associated to neurodegenerative disorders, including Alzheimer's disease. Even though traditionally Alzheimer´s disease has been associated to Aβ deposits and intracellular neurofibrillary tangles of hyperphosphorylated Tau, several studies show that neuroinflammation may be the initial cause that triggers degeneration. Recently, several studies focused on natural supplements to improve brain insulin sensitivity and reduce neuroinflammation as prevention/ therapeutic intervention to ameliorate cognitive decline. In our study, using a triple transgenic mouse model of Alzheimer's disease, we have shown that the phytohormone abscisic acid, (also an endogenous hormone), a PPARγ agonist rescue memory impairment and neuroinflammation markers (pro-inflammatory cytokines) in this mice model. Moreover, ABA can rescue BDNF and insulin receptor substrates expression. Altogether this result indicates that ABA maybe a potential treatment for syndromes of neuroinflammation etiology via the modulation of several genes involved in inflammation, insulin sensitivity and neurotrophic factors expression.

NEW APPROACHES OF BIASED AGONISM FOR POTENTIALLY NEUROPROTECTIVE CB_2R LIGANDS

Rebecca Ferrisi [1], Francesca Gado [1], Beatrice Polini [2], Kawthar A. Mohamed [3], Lesley A. Stevenson [4], Roger G. Pertwee [4], Robert B. Laprairie [3], Grazia Chiellini [2], Clementina Manera [1]

[1] *Department of Pharmacy, University of Pisa, Pisa, Italy.*
[2] *Department of Pathology, University of Pisa, Pisa, Italy.*
[3] *College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon SK Canada.*
[4] *School of Medicine, Medical Sciences and Nutrition, Institute of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, UK.*

In the last decades, cannabinoid receptor 2 (CB_2R) has continued to receive attention as a key therapeutic target in neuroprotection. Indeed, several findings highlight the neuroprotective effects of CB_2R through suppression of both neuronal excitability and reactive microglia. Generally, CB_2R agonists are not functionally selective for divergent signaling pathways, and this feature may be responsible for adverse on-target effects. An important alternative to the therapeutic development of agonists has been the development of positive allosteric modulators (PAMs), that display target subtype selectivity (i.e. ligand bias) and act by increasing agonist potency and efficacy. Signaling bias could also offer the potential to enhance the CB_2R function in neuroprotection. With the aim of combining the advantages of orthosteric and allosteric ligands, namely high CB_2R affinity and ability to induce biased signaling, in the present work we designed novel bitopic/dualsteric CB_2R ligands, structurally characterized by the presence in the same molecule of an orthosteric unit chemically connected to an allosteric pharmacophoric unit. In particular, the choice of the pharmacophore portion able to bind the orthosteric site was inspired by the class of the 1,8-naphthyridin-2(1*H*)-one-3 carboxamide derivatives, previously identified as unbiased CB_2R orthosteric agonists. The second pharmacophoric portion was derived from the CB_2R PAM analog EC21a, previously identified by us. Functional assays revealed that most of these compounds displayed a significant bias towards the activation of cAMP pathway over the recruitment of β-arrestin. Moreover, the best compounds were also evaluated for their ability to modulate cytokines production in human HMC3 microglial cells.

* *rebecca.ferrisi@phd.unipi.it*

Potential neuroprotective effect of Spent Coffee Ground extracts to counteract neurodegeneration

Michela Freschi[1], Simone Angeloni[2], Pasquale Marrazzo[1], Daniela Beghelli[3], Giovanni Caprioli[2], Gianni Sagratini[2], Silvana Hrelia[1], Cristina Angeloni[2]

[1] *Department for Life Quality Studies, Alma Mater Studiorum University of Bologna, Rimini 47921, Italy*
[2] *School of Pharmacy, University of Camerino, Via Sant' Agostino 1, I-62032 Camerino (MC), Italy*
[3] *School of Biosciences and Veterinary Medicine, University of Camerino, Via Gentile III da Varano, 62032 Camerino (MC), Italy*

Spent coffee grounds (SCGs), waste products of coffee industry, are a source of nutraceutical compounds, such as phenols, whose positive effects in counteracting neurodegeneration have been highlighted by several studies. Although neurodegenerative diseases have a multifactorial etiology, oxidative stress and neuroinflammation play a crucial role in their onset.
The aim of this study was to evaluate the antioxidant and anti-inflammatory activity of 4 different SCGs extracts in neuronal (SH-SY5Y) and microglial (BV2) cell cultures.
Cell viability was assessed by MTT assay, reactive oxygen species by DCFH-DA assay, protein expression by cytofluorimetric and immunoblotting analysis and gene expression by RT-PCR.
Data showed that the methanol extract was the most effective to protect neuronal cells from H_2O_2-induced oxidative stress through the up-regulation of the main endogenous antioxidant enzymes. The aqueous extract significantly reduced the expression of proinflammatory mediators through the modulation of the TLR4/NF-kB pathway, demonstrating to be the most effective to counteract LPS-induced neuroinflammation on the microglial cell line. Interestingly, although ethanol:H_2O and methanol:H_2O extracts were the richest in phenols, they were less effective in reducing oxidative stress and inflammation.
In conclusion, SCGs can be considered a valuable source of nutraceutical compounds to prevent/counteract neurodegeneration.

Acacia Catechu Willd. protects human SH-SY5Y cells and rat brain slices from oxidative stress-induced damage

Maria Frosini[1], Elda Chiaino[1], Roberto Stella[2], Caterina Peggion[3], Matteo Micucci[4], Roberta Budriesi[4], Massimo Valoti[1]

1 Dipartimento di Scienze della Vita, Università di Siena, Siena (Italy);
2 Dipartimento di Chimica, Istituto Zooprofilattico Sperimentale delle Venezie, Padova, (Italy)
3 Dipartimento di Scienze Biomediche, Università di Padova, Padova (Italy)
4 Dipartimento di Farmacia e Biotecnologie, Alma Mater Studiorum - Università di Bologna, Bologna (Italy)

Oxidative stress (OS) and the resulting in reactive oxygen species (ROS) generation and inflammation plays a pivotal role in the neuronal loss occurring in neurodegenerative diseases. Therefore, promising future drugs that would prevent or slow down the progression of neuro-degeneration should possess potent radical-scavenging activity. *Acacia catechu* Willd. heartwood extract (AC), already characterized for its high catechin content, is endowed of antioxidant properties. The aim of the present study was to assess AC neuroprotection in both human neuroblastoma SH-SY5Y cells and rat brain slices treated with hydrogen peroxide. In SH-SY5Y cells, AC reverted the decrease in viability as well as the increase in sub-diploid-, DAPI positive-cells, reduced ROS formation, recovered the mitochondrial potential and caspase-3 activation. AC related neuroprotective effects occurred also in rat brain slices as a reversal in the expression of the main proteins involved in apoptosis and in signaling pathways related also to calcium homeostasis following OS-mediated injury was demonstrated. Additionally, unbiased quantitative mass spectrometry allowed to assess that AC partially reverted the hydrogen peroxide-induced altered proteome, including proteins belonging to the synaptic vesicle fusion apparatus. In conclusion, the present results suggest the possibility of AC as a nutraceutical useful in preventing neurodegenerative diseases.

TRPV1 channels as putative targets in the cannabinoid-mediated synaptic activity of hippocampal neurons

Giuditta Gambino[1], G. Giglia[1], D. Gallo[1], G. Ferraro[1] and P. Sardo[1]

[1] *Department of Biomedicine, Neuroscience and Advanced Diagnostics (BIND), University of Palermo*

Endocannabinoids (eCBs) play a critical part in pathophysiological conditions rooted on neuronal excitability such as epilepsy. eCBs seem to be involved in neuroprotection, putatively acting on the cannabinoid receptor type 1 (CB1r), but also on Transient Receptor Potential Vanilloid type 1 channels (TRPV1). Indeed, CB1r and TRPV1 are involved in the transduction of stimuli at synaptic level, though exact molecular mechanisms are far from being unveiled. Thus, we aimed to investigate the role of CB1r/TRPV1 interplay in the rat hippocampal neurotransmission by whole-cell patch clamp technique to evaluate excitatory bioelectric activity in the CA1. We pharmacologically manipulated this pathway with anandamide (AEA), CB1r and TRPV1 agonist, capsaicin (CAP), TRPV1 agonist and capsazepine (CPZ), TRPV1 antagonist. Our data show that drug application significantly modifies synaptic activity by influencing action potentials parameters and mini excitatory post-synaptic currents (mEPSC). In particular, CPZ increased mEPSC amplitude, whereas CAP reduced it. As for the implication of presynaptic terminal, our results revealed that CPZ decreased mEPSC frequency and CAP increased it, also modifying in accordance the cumulative probability of inter-event intervals. Interestingly, AEA co-administration with CPZ reverts its effects, whilst CAP activity is potentiated in co-administration with AEA. Our preliminary results support the hypothesis that CB1r/TRPV1 could modulate excitatory neurotransmission ultimately improving synaptic efficiency at various levels. In this light, TRPV1 emerge as possible targets in the cannabinoid neuroprotective modulation of hippocampal bioelectric activity.

Sulforaphane as neuroprotective compounds under hypoxic and normoxic conditions.

Graziosi A., Sita G., Hrelia P., Morroni F.

Alma Mater Studiorum – University of Bologna, Pharmacy and BioTechnology, Bologna, Italy

Glioblastoma multiforme (GBM) is the most prevalent and aggressive primary brain tumor. The median survival rate from diagnosis ranges from 15 to 17 months because the tumor is resistant to the most therapeutic strategies. GBM exhibits microvascular hyperplasia and pronounced necrosis triggered by hypoxia. Several studies have indicated the chemopreventive and chemotherapeutic activities of sulforaphane (SFN), an isothiocyanate derived from cruciferous vegetables, known to inhibit cell proliferation, by provoking cell cycle arrest, and leading to apoptosis in many cell lines. In this study, we investigated the neuroprotective effects of SFN in GBM cells under normoxic and hypoxic conditions. Cell viability assays, flow cytometry, and Western blot results revealed that SFN could induce apoptosis of GBM cells in a dose-dependent manner, under both conditions. Our results demonstrated that SFN induced cell-cycle arrest and inhibited cell growth in GBM cells also under hypoxic condition and these effects may be due in part to its ability to increase oxidative status and phosphorylation of extracellular signal-regulated kinases (ERKs). Overall, we hypothesized that SFN treatment might serve as a potential neuroprotective and therapeutic strategy, alone or in combination, against GBM or other neurodegenerative disease that involves oxidative stress, alteration of cell cycle and MAPK pathway dysregulation.

Targeting Glycogen Synthase Kinase 3β to combat tauopathy in Alzheimer's disease - structure-activity relationship of novel GSK-3β inhibitors.

Izabella Góral, Tomasz Wichur, Dawid Panek, Justyna Godyń, Anna Więckowska

Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland

Alzheimer's disease (AD) is an incurable form of dementia with a vast social and economic impact. Among many factors contributing to neurodegeneration in AD, deposits of neurofibrillary tangles (NFTs) are of special interest. NFTs consist of hyperphosphorylated tau protein oligomers and exert neurotoxic effects. Under normal conditions, tau protein is responsible for the stabilization of neuronal microtubules, facilitating the process of axonal transport. In the course of AD, the activity of the tau-phosphorylating enzyme – GSK-3β – is elevated, leading to the hyperphosphorylation of tau and thus the loss of its function. Therefore the inhibition of GSK-3β may be regarded as an interesting approach in the search for effective anti-AD treatment [1].

In our research, with the support of molecular modeling techniques, we designed and then synthesized a series of novel GSK-3β inhibitors based on the *N*-(pyridin-2-yl)carboxamide scaffold. The results of *in vitro* study (Kinase-Glo luminescence assay) allowed for selection of lead structure with GSK-3β IC_{50} of 10 nM, establishment of structure-activity relationship and identification of the key structural elements responsible for high inhibitory activity. Due to the promising results of the biological assay and improved physicochemical properties of compounds we consider them as an excellent starting point for further development and optimization.

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Neuroprotective activities of the histamine H_3 receptor ligand E243 – potential treatment for Alzheimer's disease

Ewelina Honkisz-Orzechowska[1], Holger Stark[2], Katarzyna Kieć-Kononowicz[1], Dorota Łażewska*[1]

[1] *Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University Medical College in Kraków, Medyczna 9, 30-688 Kraków, Poland*
[2] *Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Universitaetsstr. 1, 40225 Duesseldorf, Germany*

One of the interesting therapeutic approaches for Alzheimer's disease (AD) treatment is the combination of acetyl- (AChE) and butyrylcholinesterase (BuChE) inhibition with additional pharmacological properties, *e.g.*: Aβ anti-aggregating activity, BACE1 inhibition, or histamine H_3 receptor (H_3R) antagonism. Moreover, the neuroprotective activity of ligands can increase pharmacological effectiveness by preventing loss of neurons and slowing disease progression.

Recently, we have described multi-target compounds against AD [1]. One of the most prominent compounds was **E243** (6-methyl-2-(4-(6-(piperidin-1-yl)hexyloxy)phenyl)-4*H*-chromen-4-one), which displayed submicromolar inhibitory activities toward all targets investigated (human H_3R K_i = 228 nM; human AChE IC_{50} = 360 nM; *electric eel* AChE IC_{50} = 501 nM; *equine* BuChE IC_{50} = 758 nM). In continued profiling, we investigated the cytotoxicity and neuroprotective activities of **E243** in neuroblastoma SH-SY5Y cells and murine microglial BV-2 cells. Treatment for 24h with an increasing concentration of **E243** showed an IC_{50} value of 24.30 μM (SH-SY5Y cells) and 53.46 μM (BV-2 cells) in MTS assay. *In vitro* neuroprotective activity against H_2O_2 (300 μM) and okadaic acid (50 nM) was assessed by DCFH-DA assay. The pre-treatment with **E243** (at 1 μM concentration) significantly prevented ROS production almost by 2-fold compared to the neurotoxins-treated cells. Moreover, we used LPS-treated BV-2 cells - the functional *in vitro* model of neuroinflammation – to assess the level of nitrate (NO) by the Griess reagent. The results showed that NO production was decreased by **E243**.

Thus, our studies indicate additional neuroprotective and anti-inflammatory properties of **E243**.

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ENNEADI: European Network in Nutritional Education for Acquired Disabilities

Marco Malaguti, Maria Cristina Barbalace, Silvana Hrelia

Dept for Life Quality Studies, University of Bologna ITALY

ENNEADI is a transnational project co-financed by the EU Programme Erasmus + KA2 – Strategic Partnerships for Vocational Education and Training in the framework of the call EAC/A02/2019 (Call for Proposals 2020). The ENNEADI project is addressed to professionals who work and assist people with neurological acquired disabilities, such as traumatic brain or spinal cord injury. The "sedentary" condition of these subjects requires special attention to healthy behaviors and good lifestyles related to nutrition, in order to prevent diseases such as obesity, diabetes, cardiovascular diseases. Moreover, TBI subjects undergo chronic neuroinflammation that lead to neurodegenerative diseases much more easily than in the general population. For this reason it is important to raise awareness and make the above mentioned professionals more aware of the importance of their role towards the quality of life and good eating practices of these non-self-sufficient subjects.
The project foresees the preparation of 4 Intellectual Outputs:
-Guidelines on training and educational methodologies in favor of professionals ordinarily working with people with acquired disability.
-Design and development of a course addressed to those professionals on dietary habits and quality of life.
-Development of an e-Learning course addressed to those professionals on preventive dietary habits;
-Policy recommendations that contribute to the promotion of structured training modules on healthy and correct eating habits for people with acquired disabilities.
Lead Applicant
FUTURA SOC CONS RL (ITALY)
Partnership
University of Bologna–Department for Life Quality Studies (ITALY)
Nueva Opción, Acquired Brain Injury Association of Valencia (SPAIN)
Vilnius University Siauliai Academy (LITHUANIA)

Consumption of tart cherries modulates cholinergic pathway in high fat diet fed rats

Ilenia Martinelli[1], Seyed Khosrow Tayebati[1], Maria Vittoria Micioni Di Bonaventura[1], Proshanta Roy[2], Vincenzo Bellitto[1], Francesco Amenta[1], Carlo Cifani[1], Daniele Tomassoni[2]

[1] *School of Pharmacy, University of Camerino, Camerino, Italy*
[2] *School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy*

Cognitive injury is associated with obesity. We investigated the controversial involvement of cerebral cholinergic markers in diet-induced obesity rats (DIO) after exposure to a high-fat diet (HFD) compared to the rats fed with a standard diet (CHOW). The effects of tart cherries seeds powder (DS) and seeds powder plus tart cherries juice (DJS) were also explored. Immunochemical and immunohistochemical results showed an upregulation of choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT) in obese rats, except for VAChT in the frontal cortex. The acetylcholinesterase (AChE) enzyme and alpha 7 nicotinic acetylcholine receptor (α7nAChR) were downregulated after HFD in the frontal cortex and hippocampus. Tart cherries supplementations enhanced ChAT and VAChT expressions in the brainstem in both DS and DJS groups. Unlike AChE was reduced in the frontal cortex and hippocampus. The juice intake led to increased α7nAChR in the brain areas compared to DIO rats. Based on the positive modulation of acetylcholine synthesizing enzyme and negative regulation of its hydrolyzing enzyme, it is possible to hypothesize that there is an attempt of recovery to increase the acetylcholine content, and the anthocyanin-rich tart cherries enhance this mechanism in specific brain areas. These data suggest how the cholinergic markers analyzed can be targeted by this nutritional supplementation. Since the activation of α7nAChR induces neuroprotection resulting from anti-inflammatory effects, tart cherries juice should be proposed within the diet to benefit the brain.

Insight into the influence of HDACi – Givinostat, on neuroinflammation and FKN/CX3CR1 axis after neonatal hypoxia-ischemia.

Paulina Pawelec[1], Teresa Zalewska[1], Małgorzata Ziemka-Nałęcz[1]

[1] *Mossakowski Medical Research Institute, Polish Academy of Sciences*

One of the determining pathogenic factors in perinatal brain damage is inflammation, caused mainly by microglia activation. Interestingly, recent data shows that histone deacetylase inhibitor (HDACi), Givinostat (ITF 2357), provides protection associated with reduction of inflammation in stroke model of adult rats. Moreover, within the CNS, fractalkine (FKN, CX3CL1) acts by interaction with microglial receptor CX3CR1 and regulates microglia activation in response to brain injury or inflammation.
Therefore in the presented study we examine the effect of Givinostat on glial cell culture after inflammatory conditions as well as on microglia-fractalkine interactions in the brain after hypoxia-ischemia.
In *in vitro* model primary mixed glial cultures were prepared from postnatal day 1 rats. Than cells were stimulated with bacterial lipopolisaccharides (LPS), Givinostat was added to the culture medium together with LPS to investigate the anti-inflammatory effect of Givinostat.
In *in vivo* experiments seven-day-old rat pups were used to unilateral carotid artery ligation followed by 60 min of hypoxia (7.6% O_2). Givinostat (10 mg/kg b.w.) was administered in a 5-day regime with the first injection given immediately after hypoxic exposure. To determine the level of fractalkine and its receptor CX3CR1 immunohistochemical analyzes were done. The influence of Givinostat on the level of CX3CL1 and CX3CR1 was evaluated by immunohistochemistry 7 and 14 days after the insult.
Givinostat has shown anti-inflammatory effect in an *in vitro* model. The obtained data suggest a disturbance in the microglia-neurons interaction.

α-LINOLENIC ACID, A NUTRACEUTICAL WITH PLEIOTROPIC PROPERTIES THAT TARGETS ENDOGENOUS NEUROPROTECTIVE PATHWAYS TO PROTECT AGAINST ORGANOPHOSPHATE NERVE AGENT-INDUCED NEUROPATHOLOGY

Tetsade Piermartiri,[1] Hongna Pan,[2] Taiza H. Figueiredo,[3] and Ann M. Marini[2]

[1] *Molecular and Cellular Biology Graduate School Program, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, USA;*
[2] *Department of Neurology and Program in Neuroscience, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, USA;*
[3] *Department of Anatomy, Physiology and Genetics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, USA;*
**Correspondence: Tetsade Piermartiri currently address is Biochemistry Department, Universidade Federal de Santa Catarina, Florianopolis, SC – Brazil Email: tetsade@yahoo.com.br*

α-Linolenic acid (ALA) is a nutraceutical found in vegetable products such as flax and walnuts. The pleiotropic properties of ALA target endogenous neuroprotective and neurorestorative pathways in brain and involve the transcription factor nuclear factor kappa B (NF-κB), brain-derived neurotrophic factor (BDNF), a major neuroprotective protein in brain, and downstream signaling pathways likely mediated via activation of TrkB, the cognate receptor of BDNF. In this review, we discuss possible mechanisms of ALA efficacy against the highly toxic OP nerve agent soman. Organophosphate (OP) nerve agents are highly toxic chemical warfare agents and a threat to military and civilian populations. Once considered only for battlefield use, these agents are now used by terrorists to inflict mass casualties. OP nerve agents inhibit the critical enzyme acetylcholinesterase (AChE) that rapidly leads to a cholinergic crisis involving multiple organs. Status epilepticus results from the excessive accumulation of synaptic acetylcholine which in turn leads to the overactivation of muscarinic receptors; prolonged seizures cause the neuropathology and long-term consequences in survivors. Current countermeasures mitigate symptoms and signs as well as reduce brain damage but must be given within minutes after exposure to OP nerve agents supporting interest in newer and more effective therapies. The pleiotropic properties of ALA result in a coordinated molecular and cellular program to restore neuronal networks and improve cognitive function in soman- exposed animals. Collectively, ALA should be brought to the clinic to treat the long-term consequences of nerve agents in survivors. ALA may be an effective therapy for other acute and chronic neurodegenerative disorders.

Effects of natural Astaxanthin on lipid peroxidation linked to ferroptosis in a human neuroblastoma cell model

Cecilia Prata[1], **Nicola Rizzardi**[1], Laura Pezzolesi[2], Chiara Samorì[3], Federica Senese[1], Walter Pitacco[3], Romana Fato[1], and Christian Bergamini[1]

[1] *Department of Pharmacy and Biotechnology; Alma Mater Studiorum-University of Bologna, Bologna, Italy. nicola.rizzardi2@unibo.it; federica.senese@studio.unibo.it; cecilia.prata@unibo.it; roma-na.fato@unibo.it; christian.bergamini2@unibo.it*

[2] *Department of Biological, Geological, and Environmental Sciences; Alma Mater Studiorum-University of Bologna, Campus Ravenna, Italy. laura.pezzolesi@unibo.it*

[3] *Department of Chemistry "G. Ciamician", Alma Mater Studiorum-University of Bologna, Campus Ravenna, Italy. chiara.samori3@unibo.it*

Astaxanthin, a red orange xanthophyll carotenoid produced mainly by microalgae, is endowed with potent antioxidant properties [1]. Due to its highly lipophilic nature, it is particularly protective against lipid peroxidation, therefore the aim of this study is to assess the potential role of Astaxanthin in counteracting ferroptosis. Ferroptosis is a recently discovered process of programmed cell death characterized by iron-dependent lipid peroxidation and is linked to the onset and development of degenerative diseases [2]. We assessed the antioxidant activities of natural astaxanthin by means of different approaches in a neuroblastoma cell model and the results showed that, when compared to synthetic astaxanthin, it is more active and bioavailable. Moreover, we induced ferroptosis in the/a SHSY-5Y cell line by means of RSL3 and Erastin, which through two different mechanisms deplete cells of the antioxidant molecule glutathione, resulting in a reduction in cell antioxidant capacity and, consequently, cell death.
The promising results obtained suggest a potential role for Astaxanthin in preventing /co-treating pathologies characterized by oxidative stress and ferroptosis, such as many neurodegenerative diseases, by means of a sustainable approach.

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Neuroprotective activity of B. jararaca snake venom peptide fractions against H2O2-induced oxidative stress is based on L-arginine production

Halyne Queiroz Pantaleão[1], Marcela Bermudez Echeverry[2] and **Carlos Alberto-Silva**[1]

[1] Natural and Humanities Sciences Center (CCNH), Experimental Morphophysiology Laboratory, Federal University of ABC (UFABC), 09606-070, São Paulo, Brazil; [2] Center for Mathematics, Computation and Cognition (CMCC), Federal University of ABC (UFABC), 09606-070, São Paulo, Brazil.

Bothrops jararaca (Bj) snake venom has a complex mixture of proteins and peptides with fascinating biological effects. Its peptide fraction (PF) increase the survival of hippocampus cells against H_2O_2-induced apoptosis by reducing oxidative stress and associated indicators. Here, we evaluated the neuroprotective activity of PF on the L-arginine metabolism in H_2O_2-induced cytotoxicity in neuronal PC12 cells. Cells (5 x 10^3 cells per well in a 96-well plate) were pre-treated at 37°C for 4 h with PF (25 to 0.04 $\mu g.mL^{-1}$), and then was followed by the addition of H_2O_2 (1.5 mM) for 20 h. The neuroprotective effects of PF were analyzed by integrity cell, mitochondrial metabolism level and reactive oxygen species (ROS) production. PF at 0.78 $\mu g.mL^{-1}$ increased integrity cell (113.6 ± 6.3%) and metabolism (96.3 ± 10.3%) against H_2O_2-induced neurotoxicity (75.6 ± 5.8 %; 66.5 ± 3.3%, respectively), reducing significantly ROS production. We also investigated the role of argininosuccinate synthetase (AsS) activity in the PF neuroprotective effects, using AsS specific inhibitor (α-Methyl-DL-aspartic acid; MDLA). AsS catalyzes argininosuccinate formation which is cleaved by argininosuccinate lyase, increasing L-arginine bioavailability. Agmatine and polyamines (spermine, spermidine, and putrescine) products from L-arginine metabolic pathways were reported as neuroprotective compounds. Interestingly, our results showed that MDLA attenuated the neuroprotective activity of PF against oxidative damage-induced cell death. Overall, this is the first study to show that PF's neuroprotective mechanism is likely based on an increase in L-arginine bioavailability caused by AsS activity in neuronal PC12 cells under H_2O_2-induced oxidative stress.

S-adenosylmethionine supplementation exerts neuroprotection through miR-29a modulation and DNA methylation

Tiziana Raia[1], Daniele Antinori[1], Mariano Bizzarri[1], Marco Lucarelli[1,2], Andrea Fuso[1]

[1] *Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy*
[2] *Pasteur Institute Cenci Bolognetti Foundation, Sapienza University of Rome, Rome, Italy*

Alzheimer's Disease (AD) is the most common cause of dementia in the elder population. *PSEN1*, an AD-related gene encoding for one of the enzymes responsible for β-amyloid production, is regulated by epigenetic mechanisms. We previously demonstrated that *PSEN1* promoter results hypomethylated both in AD patients and in transgenic TgCRND8 mice with AD-like phenotype. Its methylation profile can be restored to a physiological state by S-adenosylmethione (SAM)-supplementation. SAM is the main endogenous methyl-donor, which has a pilot role in the one-carbon metabolism, in the maintenance of different substrate's methylation status. Currently, it is known that a complex interplay between microRNAs and methylation, exists. In this study we demonstrate in cellular and animal models that miR-29a, which is involved in AD, is up-regulated by SAM-supplementation and down-regulated by hypo-methylating conditions that seems to characterize AD patients miR-29a is also downregulated in post-mortem human brain samples. Furthermore, miR-29a, specifically target BACE1, another enzyme involved in the amyloidogenic process and down-regulated by SAM, but not directly regulated by methylation. So we can argue that miR-29a is the mediator of the methylation-associated modulation of BACE1 expression. Studies of gain and loss of function of this miR are going on to ascertain this epigenetic cooperation. In conclusion, SAM and miR-29a seem to interact with a protective role for AD patients. Moreover, miR-29a holds the potential to be further studied as a innovative biomarker and a therapeutic target for nutritional based interventions.

Design and synthesis of new CK1δ protein kinase inhibitors

Andrea Spinaci,[1] Catia Lambertucci,[1] Stephanie Federico,[2] Giampiero Spalluto,[2] Davide Bassani,[3] Stefano Moro,[3] Vittoria Colotta,[4] Rosaria Volpini.[1]

[1] *School of Pharmacy, Medicinal Chemistry Unit, University of Camerino, Via S. Agostino 1, 62032 Camerino, Italy*
[2] *Department of Chemical and Pharmaceutical Science, University of Trieste, Via Licio Giorgeri 1,34127 Trieste, Italy*
[3] *Department of Pharmaceutical and Pharmacological Sciences, Molecular Modeling Section, University of Padua, Via Marzolo 5, 35131- Padua, Italy*
[4] *Department of Neuroscience, Drug Research and Child Health (NEUROFARBA), University of Florence, P.zza S.Marco, 4 - 50121 Firenze*

In recent studies it has been demonstrated the key role of the casein kinase (CK) in the development of different neurodegenerative diseases. CK are divided in two big families CK1 and CK2. In particular, in the brain of Alzheimer's patients, CK1δ isoform is present in high concentrations evoking a hyper-phosphorylation of the tau protein, causing its dissociation from the microtubules, with consequent their destabilization and inducing neuronal death. Furthermore, the CK1δ and other isoforms appear to be involved in the formation of Lewy bodies thus demonstrating a possible involvement also in the development of Parkinson's disease. The aim of this work was to obtain new of CK1δ inhibitors endowed with purine scaffold potentially able to interact at the ATP-binding site of the enzyme. Therefore, in a preliminary evaluation of some in-house compounds, the adenine bearing a cyclopentyl ring in 9 position was found able to inhibit the enzyme up to 61% of its activity, when tested at 40 μM. Hence, a new series of 9-substituted adenine derivatives were designed and synthetized starting from the commercially available 2,6-dichloropurine introducing different substituents in 2, 6 and 9 positions. The derivatives were tested to evaluate the inhibition of the enzyme activity at fixed concentration of 40 μM, then, in case the residual enzymatic activity was less than 50% were also tested at 10 μM and IC_{50} was determined.
The new derivatives were found to be inhibitors of the CK1δ enzyme with activities ranging from 99% to 58%.

Is a 1-(3-(4-(*tert*-butyl)phenoxy)propyl)-2-methylpyrrolidine a new potential drug for treatment of Parkinson's disease? In vivo preliminary evaluation

Anna Stasiak[1], Anna Wiktorowska-Owczarek[2], Marta Jóźwiak-Bębenista[2], Agata Doroz-Płonka[3], Agata Siwek[4], Katarzyna Kieć-Kononwicz[3], Dorota Łażewska[3]

[1] *Department of Hormone Biochemistry, Medical University of Lodz, Zeligowskiego 7/9 Str., 90-752 Lodz, Poland;*
[2] *Department of Pharmacology and Toxicology, Medical University of Lodz, Zeligowskiego 7/9 Str., 90-752 Lodz, Poland;*
[3] *Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University Medical College in Kraków, Medyczna 9 Str., 30-688 Kraków, Poland;*
[4] *Department of Pharmacobiology, Faculty of Pharmacy, Jagiellonian University Medical College in Kraków, Medyczna 9 Str., 30-688 Kraków, Poland.*

Parkinson's disease (PD) is a complex neurodegenerative disease of unknown etiology. Currently available treatments mainly affect alterations in appropriate neurotransmitter systems and offer temporary improvement of motor dysfunctions without modification of disease progress. New therapeutic strategies comprise multi-target drugs.
The aim of the study was the preliminary *in vivo* evaluation of the newly synthesized compound 1-(3-(4-(*tert*-butyl)phenoxy)propyl)-2-methylpyrrolidine (DL123) with good affinity for human histamine H_3 receptor (H_3R; K_i = 25 nM) and potent inhibitory activity for human monoamine oxidase B (MAO B; IC_{50} = 4 nM). The assessment concerned the effects of DL123 on the feeding behavior of rats after its repeated peripheral injections and the influence on MAO A and B and histamine *N*-methyltransferase (HNMT) activities.
Male Wistar rats kept in metabolic cages were used for the study. DL123 (3 mg/kg bw) was given s.c. for 6 consecutive days; pitolisant (H_3R antagonist/inverse agonist) was employed as the reference drug. The feeding behavior of rats was recorded daily. HNMT and MAOs activity was determined by radioassays.
In DL123-treated group, a statistically significant decline in food consumption was noted, compared with the results obtained before drug's administration (8.61 ± 0.11 *vs.* 10.63 ± 0.37 g/100 g bw). DL123 more affected the feeding pattern than pitolisant (8.95 ± 0.14 *vs.* 10.57 ± 0.42 g/100 g bw).
DL123 in the concentration used caused more than 90% decline in MAO B activity in brain cortex, whereas MAO A activity was inhibited only by 12%. The compound did not influence HNMT activity.
The 24h MTT conversion test performed on human astrocytes cell line showed low toxicity of DL123 (IC_{50} = 229.84 μM).
Conclusions: DL123 (1) crosses BBB, (2) effectively inhibits cerebral MAO B activity, (3) showing typical effects on feed consumption for an H_3R antagonist, as well as (4) has low cytotoxicity. Therefore, further research on DL123 as a potentially effective drug in the treatment of PD should be considered.
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E-Mail: anna.stasiak@umed.lodz.pl

Chrysin protects against the neurotoxicity induced by aluminium in *in vitro* and *in vivo* models

Hericles Mesquita Campos [1], Michaeld Costa [1], Lorrane Kelle da Silva Moreira [1], Hiasmin Franciely da Silva Neri [1], Cinthia Rio Branco da Silva [2], Letizia Pruccoli [3], Fernanda Cristina Alcantara dos Santos [2], Elson Alves Costa [1], **Andrea Tarozzi** [3], Paulo César Ghedini [1]

[1] *Department of Pharmacology, Institute of Biological Sciences, Federal University of Goias, Goiania, Brazil*
[2] *Department of Histology, Embryology and Cell Biology, Institute of Biological Sciences, Federal University of Goias, Goiania, Brazil*
[3] *Department for Life Quality Studies, University of Bologna, Rimini, Italy*

Chrysin is a flavonoid found mainly in honey, passion fruit and propolis with antioxidant, anti-inflammatory and cytoprotective properties. In this study, we employed an integrated approach of *in vitro* and *in vivo* models to assess the antioxidant and neuroprotective effects of chrysin against the neurotoxicity elicited by aluminium chloride ($AlCl_3$). In this regard, recent studies suggest that chronic exposure to aluminium can promote oxidative damage leading to neuronal death in different brain regions with cognition and memory deficits. In *in vitro* model, chrysin showed the ability to counteract the early oxidative stress elicited by tert-butyl hydroperoxide, an oxidant that mimics the lipid peroxidation and Fenton reaction in presence of $AlCl_3$ as well as the late necrotic death triggered by $AlCl_3$ in neuronal SH-SY5Y cells. *In vivo* studies in a mouse model of neurotoxicity induced by chronic exposure to $AlCl_3$ then corroborated the antioxidant and neuroprotective effects of chrysin using the oral route. In particular, chrysin halted the cognitive impairment induced by $AlCl_3$ as well as normalized the acetylcholinesterase and butyrylcholinesterase activities in the hippocampus. Further, chrysin counteracted the oxidative damage, in terms of lipid peroxidation, protein carbonylation, catalase and superoxide dismutase impairment, in the brain cortex and hippocampus. Lastly, necrotic cells frequency in the same brain regions was also reduced by chrysin. These results highlight the ability of chrysin to prevent the neurotoxic effects associated with chronic exposure to aluminium and suggest its potential use as a food supplement for brain health.

Effect of the HDAC inhibitor –sodium butyrate on complement system activity in rat model of neonatal hypoxia-ischemia

Karolina Ziąbska[1], Teresa Zalewska[1], Joanna Sypecka[1], Małgorzata Ziemka-Nałęcz[1]

[1]*NeuroRepair Department, Mossakowski Medical Research Institute Polish Academy of Sciences*

Perinatal hypoxic-ischemic encephalopathy remains one of the most important causes of neonatal mortality and long-term neurological sequelae. Brain damage following hypoxia-ischemia (HI) is a complex process. One of the key pathogenic factors of perinatal brain injury is the inflammatory response. The complement system plays important role in development of inflammation. The activation of complement proteins is based on a series of enzymatic and non-enzymatic cascade reactions. Complement usually protects against infection and contributes to development of neuron synapses, promoting tissue regeneration and repair, it may also cause tissue damage and when dysregulated or overactivated. Histone deacetylase inhibitors (HDACis), by reducing the expression of proinflammatory factors, may contribute to the future development of a therapeutic approach to prevent or at least limit the effects of perinatal hypoxia. In our study we used seven-day-old Wistar rats. Cerebral HI was produced by a permanent unilateral common carotid artery ligation followed by 60 min of hypoxia (7.6% O2). HDACi - sodium butyrate (SB) (300mg/kg bw) was administered in a 5-day regime with the first injection given immediately after hypoxic exposure. In our study detailed characterization of individual proteins of the complement system after HI in the presence of SB was performed. The increased expression of certain genes of the complement system, which significantly decrease after SB treatment was noted. Also, the degree of brain damage and cytoarchitecture disturbance, with particular emphasis on synaptic connections was assessed. In animals treated with SB, most of the examined tissue showed normal morphology of neurons and synaptic connections.
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