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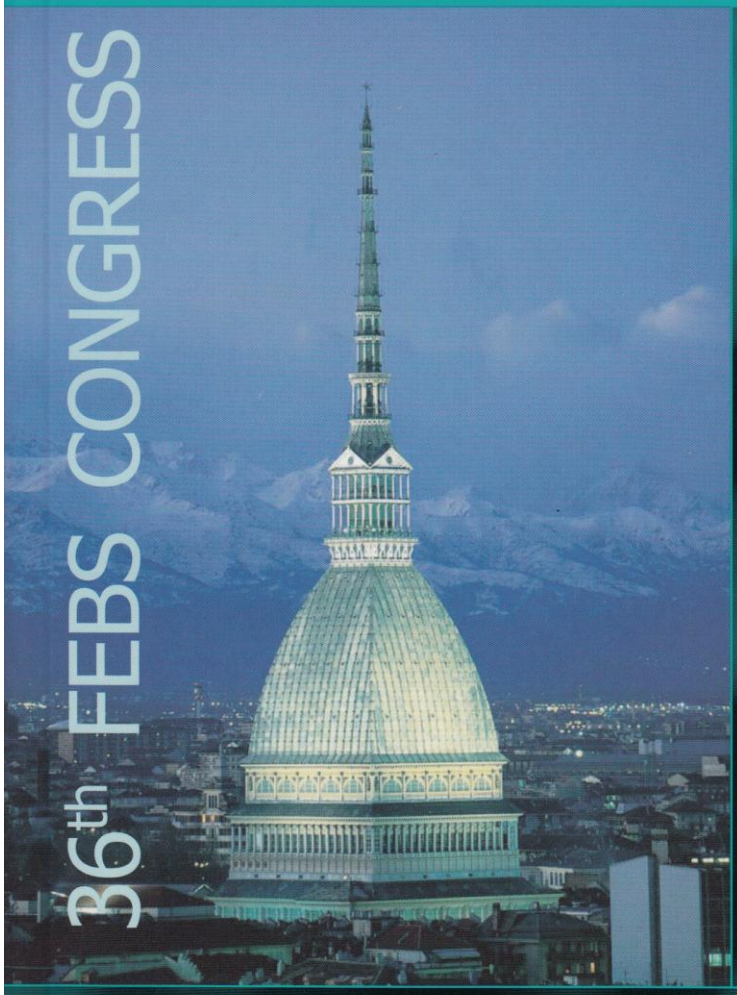

Abstracts

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model of Alzheimer's disease has been developed, in which the interaction between the α-secretase, ADAM10, and its scaffold protein, SAP97, is disrupted using a cell-penetrating Tat-peptide fused to ADAM10 proline-rich domains (Tat-Pro). Tat-peptide is able to shift the metabolism of APP towards amyloidogenesis, reproducing the initial phases of sporadic Alzheimer's disease (Epis, 2010). In this context, we investigated alterations of the calcium signaling in primary co-cultures of rat hippocampal neurons and glial cells treated with Tat-Pro peptide. Some key components of calcium signalling, such as the Group I metabotropic glutamate receptors (mGluR1 and mGluR5) and the neuronal type IP3 receptor (IP3R1) were significantly increased after the treatment with Tat-Pro, with a consequent deregulation of calcium signalling in these cells. We noticed the involvement of astroglial cells in the first phases of this pathology, showing that probably these changes appeared first in glial cells, which then affect the neurons. In this regard, we confirmed the possibility for glial cells to produce Aβ and we verified their impact on the survival of the neurons themselves. Moreover, direct application of Aβ mimicked the effect of Tat-Pro on Ca^{2+} homeostasis. FK506, a specific inhibitor of phosphatase calcineurin, was able to reduce the effects of Tat-Pro peptide, suggesting a possible involvement of this enzyme in deregulation of Ca^{2+}-related genes. Our results demonstrate that Tat-Pro model can be used for investigation of the role of alterations of calcium homeostasis in glial cells in the early stage of sporadic AD.

P14.25
Hypoxanthine and purine compounds in plasma from patients with multiple sclerosis and psoriasis

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Multiple sclerosis and Psoriasis are two autoimmune disorders in which the body's lines of defence become misguided and start damaging normal tissue. Purine nucleotide metabolism is based on three fundamental pathways: de novo synthesis, the salvage and the catabolism pathways. Several enzymes comprising the purine salvage and cleavage pathway are found with highest activity in lymphoid tissues mainly associated with T-lynphocytes. The study of hypoxanthine (HX), xanthine (X) and uric acid (UA) in plasma would provide data on the homeostasis of the purinic enzymatic system in diseases with T cell proliferation or activation. We compare the plasma concentration of adenine, guanine, HX, X and UA between patients and controls. We actually included five patients in course of relapsing-remitting Multiple sclerosis RRMS (three females and two males, median age 38 years min 18 max 67; median disease duration 3 years, min 1 max 40; median EDSS 2.5, min 1.0 max 5.0), one subject with psoriasis and 11 controls. Plasma samples were deproteinized and directly injected onto the Waters HPLC system with photodiode array detector. The mobile phase was a 40 mM potassium phosphate buffer, pH 2.2 and the optimal wavelength was 254 nm. We found in RRMS and psoriatic patients an increase of mean HX (4.31 and 10.15 μM), respectively compared to controls 2.33 μM and of mean X (1.50 and 1.20 μM), respectively compared to controls 1.10 μM. The t-area of (adenine + guanine) was higher in RRMS and psoriatic patients, but the difference was not statistically significant. Preliminary data do not confirm in RRMS and psoriatic patients a change of UA concentration to controls. These differences more than expression of an increased release of HX and X through the adenosine-to-uric-acid cascade might be the outcome of the accelerated breakdown of AMP to HX in hypoxic state with imbalance between the amount/activity of xanthine dehydrogenase/ xanthine oxidase and purine salvage pathways.

P14.26
Protective effect of lipocalin-type prostaglandin D synthase against oxidative stress-induced cell death in SH-SY5Y cells

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Lipocalin-type prostaglandin D synthase (L-PGDS) is abundantly expressed in the central nervous system and is secreted into the cerebrospinal fluid. The accumulation of reactive oxygen species (ROS) is resulted by oxidative stress and has been shown to exert neurotoxic impacts in the brain. Protein-thiol is indicated to react with ROS, and its modification is considered to be an important mechanism of biological defense. In this study, we investigated the protective effect of L-PGDS on H2O2-induced oxidative stress in human neuronal SH-SY5Y cells. Human L-PGDS has four Cys residues including two free thiols at Cys65 as a catalytic residue and Cys167, and a disulfide bond between Cys89 and Cys186. We purified two kinds of L-PGDS mutants such as C89A/C186A and C89A/C167A/C186A. By the treatment with 50 μM H2O2, the amounts of free thiols in C89A/C186A and C89A/C167A/C186A decreased to 50% and 0% of the initial amounts of them, respectively, showing that H_2O_2 reacted with the thiol of Cys65, but not that of Cys167. The MALDI-TOF MS spectrum of C89A/C167A/C186A oxidized by H_2O_2 showed an increase in the mass (ΔM = 32 Da) relative to untreated one, demonstrating that a thiol of Cys65 was oxidized to sulfinic acid. By the treatment with H_2O_2, cell viability of SH-SY5Y cells was decreased to 50%. In the presence of 5 μM C89A/C167A/C186A, however, the viability was recovered to about 70%. The suppression of L-PGDS expression by siRNA in SH-SY5Y cells decreased the viability by the treatment with H_2O_2. Further, the Kd values of untreated C89A/C167A/C186A were calculated to be 91 nM for biliverdin and 1520 nM for all-trans retinoic acid, while those of oxidized mutant were calculated to be 110 nM and 770 nM, respectively, showing that oxidized L-PGDS still had the ability to bind the ligands. These results, taken together, showed that L-PGDS could suppress the neuronal cell death by scavenging ROS at Cys65 residue without losing the function to bind hydrophobic molecules.

P14.27
The synthesis of bioconjugates of different carrier macromolecules with amyloid beta peptides in Alzheimer disease

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Conjugates of the natural and synthetic macromolecules have great importance for medicine and biotechnology with respect to