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Familial form of Alzheimer's disease (FAD) is caused by mutations in presenilin-1 (PS-1) and presenilin-2 (PS-2). PS1 and PS2 mutation are known to similar effects on the production of amyloid β peptide (Aβ) and cause of neuronal cell dath in the brain of patient of Alzheimer's disease. The importance of the alternation of cellular calcium homeostasis in the neuronal cell death by PS1 mutation in a variety of experimental systems has been demonstrated. However, no studies on the effect of PS2 or mutant PS2 on cellular calcium homeostasis, and relevance of its change to neuronal cell vulnerability against neurotoxins have been reported. In the present study, we investigated whether PS2 mutation increased vulnerability of PC12 cells and cortical neuronal cells against neurotoxic insults through perturbation of calcium homeostasis. Stable transfected PC12 cells with mutant (N1411) showed a significant increased vulnerability of cells determined by cell viability and induction of apoptosis after treatment of Aβ and L-glutamate compared to those in PC12 cells, PC12 cells expressing vector alone or expressing wild type of PS2. Similar in PC12 cell, cortical neurons from PS2 transgenic mice resulted in a greater increase of vulnerability compared to those from wide type PS2 transgenic mice. Consistent with the increased cell vnlenrability, much greater enhanced intracellular calcium level were found in PC12 cells expressing mutant PS2 after treatment of Aβ and L-glutamate. Double-labeling confocal micrograph analysis shows that ryanodine receptor (RyR) and PS2 are colocalized in endoplasmic reticulum (ER) of PC12 cells and cortical neurons from transgenic mice. PS2 and RyR expression was increased by the treatment of Aβ and L-glutamate. Moreover, pretreatment of dantrolene, an agent that block calcium release though RyR sensitive store protected against PS2 mutation-enhanced neuronal cell death. The present data suggest that PS2 mutation promotes neuronal degeneration in AD through perturbation of RyR senstive calcium homeostasis in ER.

[PA1-52] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Anti-apoptotic effect of water extract of rheum undulatum in pancreatic β-Cell, HIT-T15

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Sopungsungi-won has been used as a traditional medicine for diabetes and it has been proved evidently as a potential remedy for type 2 diabetes mellitus. Both in vivo and in vitro experiments with water extract of Sopungsungi-won have been reported to exhibit anti-diabetic effects in our previous studies. In the present study, we have chosen Rheum undulatum (RU), which is the main component of Sopungsungi-won, to examine its anti-apoptotic effect on pancreatic b-cells, HIT-T15, against oxidative stress induced by hydrogen peroxide (H_2O_2). To investigate the anti-apoptotic effect of Rheum undulatum water extract (RUWE) against H_2O_2-induced apoptosis in b-cell of pancreas, MTT assay, DAPI staining, TUNEL assay, RT-PCR and caspase-3 enzyme assay were performed in pancreatic b-cell line of hamster, HIT-T15. Through the morphological analysis, it was demonstrated that cells treated with H_2O_2 exhibit classical apoptotic features, while the occurrence of such changes was reduced in cells pre-treated with RUWE. In addition, it was shown that RUWE treated cells prior to H_2O_2 treatment induced the increase in levels of bcl-2 expression and decrease in caspase-3 enzyme activity compared to cells treated with H_2O_2 only. These results might suggest the possibility of usage of RU in patients with progressively deteriorated diabetes.

[PA1-53] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Sphingosine-1-Phosphate-Induced ERK Activation Protects Human Melanocytes from UVB-Induced Apoptosis

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Ultraviolet B (UVB) is known to induce apoptosis in human melanocytes. Here we show the cytoprotective effect of sphingosine-1-phosphate (S1P) against UVB-induced apoptosis. We also show that UVB-induced apoptosis of melanocytes is mediated by caspase-3 activation and poly(ADP-ribose) polymerase (PARP) cleavage, and that S1P prevents apoptosis by inhibiting this apoptotic pathway. We further investigated three major subfamilies of mitogen-activated protein (MAP) kinases and the Akt pathway after UVB irradiation. UVB gradually activated c-Jun N-terminal kinase (JNK) and p38 MAP kinase, while extracellular signal-regulated protein kinase (ERK) was inactivated transiently, but the Akt pathway was not affected. Blocking of the p38 pathway using SB203580 promoted cell survival and inhibited the activation of caspase-3 and PARP cleavage. These results suggest that p38 activation may play an important role in the UVB-induced apoptosis of human melanocytes. To explain this cytoprotective effect, we next examined whether S1P could inhibit UVB-induced JNK and p38 activation. However, S1P was not found to have any influence on UVB-induced JNK or p38 activation. In contrast, S1P clearly stimulated the phosphorylation of ERK, and the specific inhibition of the ERK pathway using PD98059 abolished the cytoprotective effect of S1P. Based on these results, we conclude that the activation of p38 MAP kinase plays an important role in UVB-induced apoptosis, and that S1P may show its cytoprotective effect through ERK activation in human melanocytes.

[PA1-54] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Lysophosphatidic acid Inhibits Melanocyte Proliferation via Cell Cycle Arrest

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Lysophosphatidic acid (LPA) is a well-known mitogen in various cell types. However, we were surprised to find that LPA inhibits melanocyte proliferation. Thus, we further investigated the possible signaling pathways involved in melanocyte growth inhibition. We first examined the regulation of the three major subfamilies of mitogen-activated protein (MAP) kinases and of the Akt pathway by LPA. The activations of extracellular signal-regulated protein kinase (ERK) and c-Jun N-terminal kinase (JNK) were observed in concert with the inhibition of melanocyte proliferation by LPA, whereas p38 MAP kinase and Akt were not influenced by LPA. However, the specific inhibition of the ERK or JNK pathways by PD98059 or D-JNKI1, respectively, did not restore the antiproliferative effect. We next examined changes in the expression of cell cycle related proteins. LPA decreased cyclin D_1 and cyclin D_2 level but increased $p21^{WAF1/CIP1}$ (p21) and $p27^{KIP1}$ (p27) levels, which are known inhibitors of cyclin-dependent kinase. Our results suggest that LPA induces cell cycle arrest by regulating the expressions of cell cycle related proteins.

[PA1-55] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Induction of Apoptosis in Chinese Hamster Lung Cells by NOCF via Caspase-dependent Bax expression and Cytochorme c release.

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Carbofuran(CF) is one the most widely used carbamate pesticides in the world applied for insect and nematode control. Due to its widespred use in agriculture and households. contaminaion of food, water, and air has become serious, and consequently adverse health effects are inevitable in humans, animals, wildlife and fish, it has reported that CF alone or in combination with other carbamate insecticides influences the level of reproductive and metabolic hormones such as thyroxine and corticosterone, and results in impairment of endocrine. immun behavioral functions. we investigated the effects of NOCF on the Chinese hamster lung fibroblast(CHL) induction of apoptosis. The treatment CHL cells with NOCF caused activation of caspase-3,8,9 protease. NOCF did affect the expression of proapoptotic protein bax and bid did cause a release of mitochondria cytochrome c into cytosol. A broad-spectrum caspase inhibitor and a caspase 8-specific inhibitor completely blocked NOCF-induced activation of caspase 3 and cell death. These findings and data showing the early release of cytochrome c,