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,

expression of bax and bid suggest that mitochondria pathway is primarily involved in NOCF induced apoptosis.

[PA1-56] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Antidiabetic Activity of Formular containing an Euonymus alata and Mori Foluim in Multiple Low Dose Streptozotocin-induced Rats

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Antidiabetic activity of formular containing an Euonymus alata (EA) and Mori Foluim (MF) was investigated in oral glucose tolerance test (OGTT) and multiple low dose Streptozotocin (MLDSTZ)-induced rats. Optimum ratio between EA and MF was found to be 1:1 in OGTT, and two strengths (250 and 500 mg/kg for each medicinal plant) were coadministered with 20 mg/kg of STZ in 5 consecutive days. At 3rd week, water and food intakes were compared between groups and polydipsia and polyphagia shown in diabetic control were markedly improved in dose dependent manner. Plasma glucose level in E2M2 (500 mg/kg for EA and MF)-treated group was significantly lowered to 153 mg/dl from 300 mg/dl in diabetic control (49% inhibition). Plasma insulin levels in E1M1 (250 mg/kg for EA and MF) and E2M2-treated groups were increased by 13% and 26%, respectively, when compared to the diabetic control, suggesting that the formular may protect the pancrease beta cell from destruction by STZ administration. Beta cell sparing effect of the formular was confirmed by HE staining of pancreata. Protein expression of glucose transporter 4 (GLUT4) was examined by Western blot analysis, and 45% reduction of GLUT4 expression in diabetic control group compared to the normal control was recovered by 2 and 3.5-fold in E1M1 and E2M2-treated groups, respectively.

[PA1-57] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Effects of (1R,9S)-(β)-Hydrastine on Intracellular Calcium Concentration in PC12 Cells

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(1R,9S)-(β)-Hydrastine (HS) at 10-50 μM has been proven to have an inhibitory effect on dopamine biosynthesis in PC12 cells by the inhibition of tyrosine hydroxylase (TH) activity and TH gene expression. In the present study, therefore, the effects of HS on the basal and K^+-induced dopamine release, and Ca^{2+} influx induced by high K^+ and caffeine in PC12 cells were investigated. The dopamine release by high K^+ (56 mM) was inhibited by co-incubation of 20 μM HS. Application of HS also significantly reduced the magnitude of the maintained Ca^{2+} influx induced by K^+ depolarization. In addition, when the cells were exposed to 2 μM nifedipine after the treatment with 50 μM HS, the reduction of $[Ca^{2+}]_i$ was continued. The reduction of basal $[Ca^{2+}]_i$ was also observed in response to HS when PC12 cells were bathed in Ca^{2+}-free KRH solution, suggesting that HS inhibits Ca^{2+} release from intracellular Ca^{2+} stores. The application of 20 mM caffeine in Ca^{2+}-free KRH solution caused a rapid rise of $[Ca^{2+}]_i$. The pretreatment with HS reduced caffeine-induced rise of $[Ca^{2+}]_i$ leading to the activation of store-operated Ca^{2+} entry. In the presence of extracellular Ca^{2+} (2.5 mM), the application of 20 mM caffeine also caused a rapid Ca^{2+} influx compared with Ca^{2+}-free condition. The application of HS after caffeine treatment also reduced the magnitude of the maintained Ca^{2+} influx induced by caffeine. When 50 μM HS was added after the treatment of 1μM thapsigargin, a slight decrease in $[Ca^{2+}]_i$ was observed in PC12 cells. These results newly suggest that HS is an inhibitor of, working on the modulation of L-type Ca^{2+}channels, Ca^{2+} release from intracellular Ca^{2+} stores and store-operated Ca^{2+} channels in PC12 cells.

[PA1-58] [2003-10-10 14:00 - 17:30 / Grand Ballroom Pre-function]

Inhibitory effects of tetrahydropapaverine on serotonin biosynthesis in murine