Sphingosine-1-phosphate Promotes the Survival of Mel-Ab Cells via ERK and Akt activation

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Sphingolipids have been emerged as bioactive lipid modulators that mediate a variety of cell functions. However, the effects of sphingolipids on the cell growth and survival of melanocytes are not yet known. In the present study, we investigated the actions of sphingolipids in Mel-Ab melanocytes. We observed the cytoprotective effect of sphingosine-1-phosphate (SPP) on UVB-induced cell death. Since SPP is well known as a mitogenic agent, it is possible that the mitogenic effect of SPP may contribute to cell survival. Surprisingly, we found that SPP inhibited DNA-synthesis significantly. We were next interested in the regulation of three subfamilies of MAPKs and the Akt pathway by SPP against UVB-induced cell death. UVB irradiation resulted in the remarkable and sustained activation of c-Jun N-terminal kinase (JNK), while p38 MAP kinase was activated transiently. The basal level of extracellular signal-regulated protein kinase (ERK) phosphorylation decreased 30 min after UVB irradiation, whereas that of Akt phosphorylation was not affected by UVB. These results suggest that JNK and p38 activation and ERK inactivation may be responsible for UVB-induced apoptosis. Therefore, we investigated whether SPP could inhibit UVB-induced JNK and p38 activation to explain its cytoprotective effect. However, SPP had no effect on UVB-stimulated JNK and p38 activity. In contrast, we clearly observed that SPP potently stimulated the phosphorylation of both ERK and Akt, which are involved in cell survival signaling cascade. Furthermore, the specific inhibition of the ERK and Akt pathways by PD98059 and LY294002, respectively, restored the cytoprotective effect induced by SPP.

[PA1-69] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Analysis of vasopressin-induced Ca2+ influx in rat hepatocytes

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To analyze vasopressin-induced Ca^{2+} influx in liver cells, rat hepatocytes were isolated and attached to collagen-coated cover slips. Using fura-2, a Ca^{2+}-sensing dye, changes in intracellular Ca^{2+} concentration by vasopressin were monitored. Results in this communication suggested that vasopressin-induced Ca^{2+} influx consists of two distinguishable components. One was present for a short time and the other was for a long time until it happened. The former influx was blocked by SK&F96365 in a dose-dependent manner. Vasopressin-induced Ca^{2+} release from internal stores diminished in a primary culture of hepatocytes according to the culture time. However, changes in vasopressin-induced Ca^{2+} influx across the plasma membrane differed from changes in the Ca^{2+} release from internal stores.

[PA1-70] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Sphingosine-1-Phosphate Decreases Melanin Synthesis via Sustained ERK Activation and Subsequent MITF Degradation

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This study shows that sphingosine-1-phosphate (SPP) significantly inhibits melanin synthesis in a concentration-dependent manner, and that the activity of tyrosinase was also reduced in SPP-treated cells. In contrast, a specific extracellular signal-regulated protein kinase (ERK) pathway inhibitor, PD98059 increased tyrosinase activity and melanin production, and PD98059 restored the reduced tyrosinase activity and pigmentation induced by SPP. We also found that SPP induces the sustained activation of ERK and the subsequent degradation of microphthalmia-associated transcription factor (MITF), which plays a key role in melanogenesis. Thus, we further studied the relationship between the ERK pathway and melanin synthesis. PD98059 was found to prevent the MITF phosphorylation and degradation induced by SPP and to abrogate reduced tyrosinase and tyrosinase-related protein 1 (TRP1) production by SPP. These results indicate that the ERK pathway is potently involved in the melanogenic signaling cascade, and that SPP-induced ERK activation contributes to reduced melanin synthesis via MITF degradation.

[PA1-71] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

A Collaborative Study to Establish a Korea National Biological Standard for Antithrombin Concentrate

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We have carried out collaborative study to evaluate a preparation of antithrombin concentrate whether or not it was suitable to serve as the candidate for a Korea National Biological Standard. Six laboratories, including three manufacturers and three National Control Laboratories, participated in this study. The potency of this candidate preparation was determined using the heparin cofactor chromogenic method. The method is described in the Minimum Requirements for Biological Products and the European Pharmacopoeia. The candidate gave excellent intra- and inter-laboratory correlations when assayed against the second international standard for antithrombin concentrate, coded as 96/520. The participants contributed data from a total of 88 assays and the results were accepted as statistically valid when the outcome of the analysis was for linearity of dose-response relationships and for intersection at a common point at zero dose in slope-ratio model. Combined potency estimates were obtained by taking geometric means of results from all assays at each laboratory, and overall potency estimates were calculated as geometric means of results from all laboratories. The results were expressed in the form of histograms and 95% confidence intervals. Based on the results of the collaborative study described here, the candidate reference standard is judged to be suitable to serve as the Korea National Biological Standard for antithrombin concentrate.

[PA1-72] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

A NAT for reliable HBV DNA Screening of Plasma

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The safety of blood and blood products is ensured by careful selection of donors, screening of