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We previously reported that a synthetic naphthoquinone analog, 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (NA), effectively induces apoptosis in human leukemic HL-60 cells. However, the cellular mechanism by which NA induces cell death remain unclear. In this study, we show that NA induces activation of capases, release of cytochrome c and upregulation of proapoptotic Bax protein. Futhermore, NA suppressed phosphorylation of Akt and Bad, suggesting that Akt regulates NA-induced apoptosis. Expression of a dominant negative Akt enhanced NA-induced apoptosis, suggesting that naphthoquinone analog induces apoptosis through activating proapoptotic pathway and by the inactivation of antiapoptotic pathway.

[PC3-3] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Decursin derivative-004 protect renal cell damage via p38 MAPK inhibition

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Hypertrophy and the alteration of renal cell growth have been reported as early abnormality in diabetic nephropathy. However, the effects of high PKCglucose and its action mechanism in renal proximal tubular cell (PTC) have not been elucidated. High glucose condition increases diacyl glycerol (DAG) and activates protein kinase C (PKC) in renal tubular cells. The PKC activates mitogen-activated protein kinases (MAPK), such as extracellular regulated kinase (ERK) and p38 MAPK. It was reported that decursin, originally known as a PKC activator, protects kidney from high glucose condition. In this study, it was elucidated that decursin derivatives down-regulates PKC alpha and blocks activation p38 MAP kinase in renal proximal tubular cells, but they does not affect on ERK signaling. Our results demonstrate that the renal protective effect of decursin derivatives against high glucose-induced damage is mediated via the inhibition of PKC dependent p38 MAPK.

[PC3-4] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Anti-angiogenic activity of conjugated linoleic acid on the basic fibroblast growth factor-induced angiogenesis

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Conjugated linoleic acid (CLA) is a potent inhibitor of mammary carcinogenesis. Cancer cells produce various angiogenic factors which stimulate host vascular endothelial cell mitogenesis and chemotaxis for their growth and metastasis. Basic fibroblast growth factor (bFGF) is a potent angiogenic factor that is expressed in many tumors. In this study, we found that CLA decreased bFGF-induced endothelial cell proliferation and DNA synthesis in a dose-dependent manner. However, CLA did not inhibit endothelial cell migration. Furthermore, CLA showed a potent inhibitory effect on embryonic vasculogenesis and bFGF-induced angiogenesis in vivo. Collectively, these results suggest that CLA selectively inhibits the active proliferating endothelial cells induced by bFGF, which may explain its anti-carcinogenic properties in vivo.

[PC3-5] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Decusinol angelate inhibits UVB-induced MMP-1 induction via Mitogen-activated Protein Kinase Pathway in human skin fibroblasts

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UV-B irradiation increases the synthesis of matrixmetalloproteinase-1 (MMP-1) that degrades skin collagen in human skin. In this work, we investigated the photoprotective effect of decursinol angelate (DEA) extracted from Angelica gigas on human skin fibroblasts. DEA inhibited UVB-induced MMP-1 induction, which was confirmed by western blot and ELISA. We examined upstream signal transduction pathway and the action mechanism of DEA on UVB induction of MMP in human skin fibroblasts. UV irradiation stimulated mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), c-Jun amino-terminal kinase (JNK), and p38. Pretreatment of DEA on human skin fibroblasts inhibited ERK, p38 and JNK phosphorylation, while DEA had no effect on reducing ROS generation. DEA inhibited the UVB-induced MMP-1 induction by regulating MAPK phosphorylation in human skin fibroblast. These results demonstrate that DEA can be used as a potent anti-aging agent.

[PC3-6] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

A new strategy for high productivity of Erythropoietin in CHO cell by introducing urea cycle enzymes

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The efficient Erythropoietin(EPO)-expression system in mammalian cells is required for massive production for therapeutic use. Ammonium ion is a major problem in the production of useful proteins by cultured animal cells and therefore it is of importance to devise a system by which a high productivity of human therapeutic recombinant protein can be maintained or enhanced under low ammonium concentration. To reduce the ammonium ion accumulated in EPO producing CHO cell(IBE), we introduced the first two enzymes of urea cycle, carbamoyl phosphate synthetase (CPS) and ornithine transcarbamoylase (OTC) into IBE using a stable transfection method. Transfectants expressing CPS and OTC were selected and confirmed by RT-PCR. IBE expressing CPSI and OTC (CO5) showed 2-2.5 times higher productivity of EPO than the parental cell, IBE. Also, CO5 had 15-25% higher cell viability and 15-20% lower ammonia concentration per cell after 96 hr culture than IBE. These results indicate that improvement of higher ammonia removal activity in CHO cell by introducing urea cycle enzymes led to enhancement of recombinant human EPO productivity with higher cell viability. Comparisons of glycosylation and bioactivity of EPO purified from IBE and CO5 is currently in progress.

[PC3-7] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

p38 mitogen-activated protein kinase (MAPK) regulates ceramide-induced apoptosis in HL-60 cells.

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Ceramide is a lipid second messenger that is involved in apoptotic cell death. In this study, we show that p38 MAPK plays an important role in the regulation of ceramide-induced apoptosis. We found that SB203580, a p38 kinase inhibitor, blocked the effects of ceramide to induce Bax translocation to mitochondria, activation of caspase-3, and DNA fragmentation. Furthermore, expression of a dominant negative form of p38 MAPK suppressed ceramide-induced Bax translocation, suggesting that p38 kinase activity is essential for Bax translocation. In contrast, LY294002, a PI3K inhibitor had little effect on Bax translocation. Expression of a dominant negative form of Akt, a downstream effector of PI3K, moderately promoted cell death by ceramide. These data show that both the p38 and Akt pathways are involved in ceramide-mediated apoptotic pathway, but Bax translocation is only governed by p38-mediated pathway.

Poster Presentations - Field D1. Medicinal Chemistry