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We have previously reported that activation of K^+-Cl^--cotransport (KCC) by N-ethylmaleimide (NEM) induces apoptosis through generation of reactive oxygen species (ROS) in HepG2 human hepatoblastoma cells. In this study we investigated the possible role of phospholipase A_2 (PLA_2)-arachidonic acid (AA) signals in the mechanism of the NEM actions. In these experiments we used arachidonyl trifluoromethylketone ($AACOCF_3$), bromoenol lactone (BEL) and *p*-bromophenacyl bromide (BPB) as inhibitors of the calcium-dependent cytosolic PLA_2 ($cPLA_2$), the calcium-independent PLA_2 ($iPLA_2$) and the secretory PLA_2 ($sPLA_2$), respectively. BEL significantly inhibited the NEM-induced KCC activation, ROS production and apoptosis, whereas $AACOCF_3$ and BPB did not. NEM increased AA liberation in a dose-dependent manner, which was markedly prevented only by BEL. The NEM-induced actions (KCC activation, ROS generation and apoptosis) were not significantly altered by treatment with indomethacin and nordihydroguaiaretic acid (NDGA), selective inhibitors of cyclooxygenase (COX) and lipoxygenase (LOX), respectively. Treatment with AA or 5, 8, 11, 14-eicosatetraynoic acid (ETYA), a non-metabolizable analogue of AA, markedly activated the KCC, produced ROS and induced apoptosis. Collectively, these results suggest that AA liberated through activation of $iPLA_2$ may mediate the NEM-induced ROS generation, KCC activation, and apoptosis induction in HepG2 cells.

[PA1-6] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Inhibitory effect of 2-amino-3-ethoxycarbonyl-1-methyl pyrolo (3,2-b) naphtho-4,9-dione on tumor cell invasion in human fibrosarcoma cells by downregulating matrix metalloproteinase-2 and 9

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Matrix metalloproteinases (MMPs) play an important role in tumor invasion and metastasis by matrix degradation. To analyze the effect of 2-amino-3-ethoxycarbonyl-1-methyl pyrolo (3,2-b) naphtho-4,9-dione (compound 1) on the invasion or metastasis of cancer cells the expression of matrix metalloproteases (MMPs) was investigateded in human fibrosarcoma HT1080 cells by RT-PCR or gelatin zymographic methods. As a result, compound 1 decreased the expression of MMP-2 and 9, but increased the expression of a tissue inhibitor of metalloproteinase-1 (TIMP-1). In addition, compound 1 inhibited cancer cell migration and colony formation. These results suggest that compound 1 might be inhibiting tumor cell invasion and metastasis by suppression of MMPs and elevation of TIMP-1 production in tumor cells, and thus compound 1 could be a lead candidate for developing anti-metastatic or anti-invasive agent against cancer cell growth. (This work was supported in part by Korea research FoundationGrant, KRF-2001-005-F00023).

[PA1-7] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

High-Throughput Screening for Novel Inhibitors of Protein-Tyrosine Phosphatase-1B

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Protein-tyrosine phosphatases (PTPs) constitute a family of receptor-like and cytoplasmic enzymes, which catalyze the dephosphorylation of phosphotyrosine residues in a variety of receptors and

signaling molecules. Thirty subtypes of PTPs have been identified in human genomes. Among PTPs, PTP1B has been suggested as a negative regulator of insulin signaling. Overexpression of this enzyme has been known as a cause of obesity and type II diabetes, so it is a target for drug discovery. However, PTPs are involved in several signaling pathways, it is possible that PTPs inhibition may give rise to unwanted side effects. Therefore, specific PTP1B inhibitors that may be free of side effects and highlight the potential of selective therapeutic efficacy in targeting PTP1B are required.
The 73,000 compounds were screened using high-throughput experimental techniques for searching compounds that inhibited PTP1B. 4-nitrophenyl phosphate assay has been used for the first assay in the format 96-well plate. Using this assay system, we have discovered 61 hit compounds. For the sencond screening, hit compounds are assayed with phosphotyrosine peptide as substrate. Finally, we test isozymes selectivity of each compounds. In this schedule, we are screening for discovering the novel drug of anti-obesity and anti-diabetes.

[PA1-8] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Growth inhibition and cell cycle phase-specific apoptosis induced by celecoxib in human NSCLC cells in vitro.

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Cyclooxygenase-2 (COX-2) is an inducible enzyme which produces prostanoids by various stimuli. Overexpression of COX-2 in many tumor types indicates its association with tumor progression, which has been a promising target for chemoprevention and chemomodulation. We studied conc- and time-dependency of COX-2 inhibition, growth inhibition, and cell cycle arrest induced by celecoxib, a selective COX-2 inhibitor, in human non-small cell lung cancer (NSCLC) A549 cells. COX-2 activity $IC_{50}$and IC_{80} for 24hr exposure were approx. 0.1 and 1μM, respectively. The inhibition increased with prolonged exposure, i.e., 20% at 6hr to 60% at 24hr when exposed to 0.1μM. Cytotoxic IC_{50} after 6hr exposure was 110μM and decreased to 20μM after 72hr exposure. These conc were about 600 fold higher than those of COX-2 inhibition. Fifty μM (cytotoxic $IC_{80,72hr}$) of celecoxib induced G1 phase arrest and apoptosis in cells in G1 phase. In summary, (1) the drug conc inducing COX-2 inhibition and cytotoxicity were different by more than 600 folds in human NSCLC cells, suggesting that these two effects may not have direct causal relationship, and (2) growth inhibition and apoptosis induced by celecoxib are associated with G1 phase arrest, which may be important in designing of combination regimen of celecoxib. Changes in expression level of COX-2 and other factors at higher conc are under investigation to elucidate the mechanism of growth inhibition by celecoxib in human NSCLC cells.

[PA1-9] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

4-Hydroxy nonenal (HNE) Induces Apoptosis and Cell Cycle Arrest in Bovine Aortic Endothelial Cells

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4-Hydroxy nonenal (HNE) is a lipid peroxidation product derived from oxidized ω-6 polyunsaturated fatty acids, such as arachidonic acid. HNE is widely used as a marker of lipid peroxidation. To study the hypothesis that HNE may induce apoptosis and cell cycle arrest, we estimated cytotoxicity of HNE