shown to inhibit experimental carcinogenesis and mutagenesis, but molecular mechanisms underlying its chemopreventive activities remain unclear. In the present work, we found that curcumin inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced expression of COX-2 in female ICR mouse skin when applied topically 30 min prior to TPA as determined by both immunoblot and immunohistochemical analyses. Multiple lines of evidence support the role of the eukaryotic transcription factor NF-κB in regulation of COX-2 expression. In agreement with this notion, the NF-κB inhibitor pyrrolidine dithiocarbamate suppressed not only NF-κB activation but also induction of COX-2 in mouse skin. Curcumin treatment attenuated TPA-stimulated epidermal NF-κB activation, which was associated with its blockade of degradation of the inhibitory protein IκBα and subsequent translocation of p65 subunit to nucleus. TPA treatment resulted in rapid activation via phosphorylation of extracellular signal-regulated protein kinase (ERK)1/2 and p38 mitogen-activated protein kinase (MAPK), which are upstream of NF-κB. The MEK1/2 inhibitor U0126 strongly inhibited NF-κB activation, while p38 MAPK inhibitor SB203580 failed to block TPA-induced NF-κB activation in mouse skin. Furthermore, U0126 blocked the TPA-induced IκBα phosphorylation by TPA, thereby blocking the nuclear translocation of NF-κB. Curcumin inhibited the catalytic activity of ERK1/2 in mouse skin. Taken together, suppression of COX-2 expression by inhibiting ERK activity and NF-κB activation may represent molecular mechanisms underlying previously reported anti-tumor promoting effects of this phytochemical in mouse skin tumorigenesis.

[PC1-18] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

p38 MAP kinase and Akt regulate Bax translocation from mitochondria during ceramide-mediated apoptosis

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Ceramide is an important lipid messenger involved in mediating a variety of cell functions including apoptosis. Previously, we have shown that ceramide induces Bax translocation which is associated with cytochrome c release from the mitochondria. In this study, we show that p38 MAP kinase is involved in ceramide-induced Bax translocation. In human leukemic cells, ceramide stimulated the phosphorylation of p38 MAP kinase. Preincubation of cells with SB203580, a specific inhibitor of p38 inhibited DNA fragmentation induced by cell-permeable ceramide. Protection from apoptosis by SB203580 inhibited activation of caspase-3 and translocation of Bax. Furthermore, expression of dominant negative mutant of p38 attenuated ceramide-induced Bax translocation and apoptosis, indicating that p38 activation is required for Bax-mediated apoptosis induced by ceramide. In respect to PI3 kinase pathway, expression of constitutively active Akt reduced cell death and Bax translocation. We also found that expression of dominant-negative p38 suppressed ceramide-induced Akt dephosphorylation, indicating crosstalk between the two signaling pathways. Our results show that both the p38 and Akt pathways are involved in ceramide-mediated apoptosis by regulating Bax translocation.

[PC1-19] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Potent inhibition of human cytochrome P450 1 enzymes by SY-081

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Recently we have reported that various hydroxystilbenes show strong inhibition of human cytochrome P450 1 enzyme activities. A series of syntheic trans-stilbene derivatives were prepared and their inhibitory potentials were evaluated with the bacterial membrane of recombinant human cytochrome P450 1A1, 1A2 and 1B1 coexpressed with human NADPH-P450 reductase to find a new inhibitor of cytochrome P450 enzymes. Of the compounds tested, SY-081 exhibited a potent inhibition of human cytochrome P450 1B1 with an IC_{50} value of 2.6 nM. SY-081 also showed the inhibition of cytochrome P450 1A1 with IC_{50} value of 47.6 nM and cytochrome P450 1A2 with IC_{50} value of 116.6 nM. SY-081 showed 18-fold selectivity for cytochrome P450 1B1 over 1A1 and 45-fold selectivity for cytochrome P450 1B1 over 1A2. We also have investigated the inhibition kinetics of cytochrome

P450 1A1, 1A2, or 1B1 by SY-081. The modes of inhibition by SY-081 were mixed-type for all three cytochrome P450 1 enzymes. The K_i values of SY-081 for P450 1A1, 1A2, or 1B1 inhibition were 15.1, 29.6, or 1.4 nM, respectively. Effect of preincubation with NADPH on inhibition of cytochrome P450 1A1, 1A2, 1B1 by SY-081 was determined. Taken together, the data suggest that SY-081 is a new potently selective inhibitor of cytochrome P450 1B1 and understanding of the detailed mechanism of SY-081 action will be helpful to elucidate how cytochrome P450 1B1 is involved in the metabolism of procarcinogens such as benzo[a]pyrene or DMBA.

[PC1-20] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Induction of cell death by 2,4,3',5'-tetramethoxystilbene in human acute promyelocytic leukemia (HL-60) cells and its mechanism.

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We have previously shown that 2,4,3',5'-tetramethoxystilbene (TMS), a synthetic trans-stilbene analogue, is one of the most potently selective inhibitor of human cytochrome P450 1B1 in vitro and in vivo. In the present studies, the apoptotic effects of TMS were investigated in HL-60 cells. The effects of TMS on the proliferation of HL-60 cells were determined with MTT assay. TMS exhibited cytotoxicity with an IC_{50} value of 37 nM. Cotreatment with TMS and etoposide, a well-known anticancer drug significantly enhanced the cytotoxicity. We have investigated the detailed mechanism of cell death by TMS. We have determined that the cytotoxic effect of TMS was due to the induction of apoptosis, which was confirmed by Annexin V staining, poly(ADP-ribose) polymerase (PARP) cleavage, and cytochrome c release. TMS also induced DNA fragmentation in a dose-dependent manner. Taken together, we suggest that the apoptosis-inducing activity and inhibitory activity of cytochrome P450 1B1 of TMS make this compound be useful for anticancer strategies of hormone-mediated carcinogenesis.

[PC1-21] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Inhibitory mechanism of cyclohexylimminobenzoxathiol LYR-64 compound on LPS-induced NO production

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Nitric oxide (NO) is known to work as an important signaling molecule involved in regulating a wide range of biological activities in the neuronal, vascular, and immune system. NO and its metabolites mediate a number of host defence functions and are also implicated in the pathogenesis of tissue damage associated with inflammation. Cyclohexylimminobenzoxathiol LYR-64 compound inhibited LPS-induced NO production in murine macrophages Raw264.7 with an IC50 value of 0.7 uM with 95.9% inhibition at 3 uM, 63.5% at 1 uM and 30.2% at 1 uM. Moreover, iNOS mRNA and its protein expressions were abrogated by the cyclohexylimminobenzoxathiol LYR-64 compound in LPS-stimulated Raw264.7 cells. To further investigate the mechanism responsible for the inhibition of iNOS gene expression by cyclohexylimminobenzoxathiol LYR-64 compound, we examined the effect of the compound on NF-kB signaling. We found that cyclohexylimminobenzoxathiol LYR-64 compound inhibited NF-kB DNA binding activity as well as nuclear translocation, but did not inhibit IkB degradation.

[PC1-22] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Inhibitory effects of [6]-gingerol on phorbol ester-induced cox-2 expression in mouse skin: p38 mapk and p65/rela as possible molecular targets

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