hydroxyl radicals. Whereas adenosine 5'-monophosphate as substrate exhibited a modest protection against the glutathione/Fe^{2+} action, a remarkable protection was expressed by divalent metal ions such as Zn^{2+} or Mn^{2+}. Structure-activity study with a variety of thiols indicates that the inactivating action of thiols in combination with Fe^{2+} resides in the free sulfhydyl group and amino group of thiols. Overall, thiols, expressing more inhibitory effect on the activity of 5'-nucleotidase, were found to be more effective in potentiating the Fe^{2+}-mediated inactivation. These results suggest that ecto-5'-nucleotidase from brain membrane is one of proteins susceptible to thiols/Fe^{2+}-catalyzed oxidation. The work was partly supported by Korea Research Foundation (1998-001-F00772).

[PC1-13] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Chemopreventive effect of capsaicin in SK-Hep-1 hepatocellular carcinoma cell line

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Hepatocellular carcinoma is one of the most lethal malignancies and there is no effective preventive measure in this highly malignant disease to date. In the present study, we investigated the chemopreventive potential of capsaicin (8-methyl-N- vanillyl-6-nonenamide), the principal pungent ingredient found in hot red pepper, in SK-Hep-1 hepatocellular carcinoma cells. Treatment of capsaicin inhibited growth of SK-Hep-1 cells in a concentration-dependent manner, with an IC_{50} value of 119 uM. Methoxy-capsaicin was less potent (IC_{50} of 264 uM), indicating that the hydroxyl group of capsaicin is important in growth-inhibitory property of capsaicin. This study reveals that the inhibitory effect of capsaicin on SK-Hep-1 cell growth is mainly due to the induction of apoptosis as evidenced by DNA fragmentation and nuclear condensation. In order to investigate the molecular mechanisms of capsaicin-induced apoptosis, we examined the effect of capsaicin on anti-apoptotic Bcl-2 and pro-apoptotic Bax levels. We show that capsaicin prominently reduced the ratio of Bcl-2 to Bax which may trigger apoptosis in SK-Hep-1 cells. We also show that caspase-3 activity may be involved in capsaicin-induced apoptosis. These results demonstrate that capsaicin efficiently induced apoptosis in SK-Hep-1 cells, suggesting an effective strategy for hepatocellular carcinoma chemoprevention.

[PC1-14] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Induction of apoptosis by 3,4'-dimethoxy-5-hydroxystilbene in human myeloid leukemic HL-60 cells

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3, 4···-Dimethoxy-5-hydroxystilbene (DMHS) is a hydroxystilbene compound obtained by methylation and acid hydrolysis of piceid (resveratrol-3-O-glucoside) from *Polygonum cuspidatum*. Herein, we report that DMHS induces programmed cell death or apoptosis in human promyelocytic leukemic HL-60 cells. We found that treatment of HL-60 cells with DMHS suppressed the cell growth in a concentration-dependent manner with IC_{50} value of 25 uM. DMHS increased the apoptosis characterized by internucleosomal DNA fragmentation and nuclear condensation. The cell death by DMHS was partially prevented by the caspase inhibitor, zVAD-fmk. DMHS caused activation of caspases such as caspase-3, -8, and -9. Immunoblot experiments revealed that DMHS-induced apoptosis was associated with the

induction of Bax expression. The release of cytochrome c from mitochondria into the cytosol was increased in response to DMHS. Taken together, DMHS leads to apoptotic cell death through a caspase-dependent mechanism. Increased Bax expression and release of cytochrome c are important to apoptotic effect of DMHS in HL-60 cells.

[PC1-15] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Possible roles of reactive oxygen species and Rac1 in capsaicin -induced apoptosis of H-ras-transformed breast epithelial cells

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Efforts have been made to develop a chemoprevention strategy that selectively triggers apoptosis in malignant cancer cells. Capsaicin(trans-8-methyl-N-vanillyl-6-nonenamide), the major pungent phytochemical in red pepper, has been recently shown to exert anti-carcinogenic or chemopreventive properties. We have previously shown that capsaicin selectively induces apoptosis of H-ras transformed human breast epithelial cells (H-ras MCF10A) in which activation of c-Jun N-terminal protein kinase-1 (JNK-1) and deactivation of extracellular signal-regulated kinases (ERKs) may be involved. Since Ras-transformed fibroblasts were shown to produce reactive oxygen species (ROS) through a mechanism which is dependent of Rac1, we wished to further investigate on the capsaicin modulation of H-ras pathway and ROS generation for better understanding the mechanism of the selective chemopreventive effect of capsaicin. Here, we show that capsaicin treatment induce ROS generation in H-ras MCF10A cells. We also show that capsaicin-induced growth inhibition was significantly inhibited in H-ras MCF10A cells expressing a dominant negative Rac1 gene product (N17 rac1). These results suggest that Rac1 may be critical to the capsaicin-induced apoptosis and that Rac1 is a downstream effector of Ras in signal transduction pathway. Effects of capsaicin on TPA-induced NF-kappaB activation in these cells are currently being investigated.

[PC1-16] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Biochemical Characteristics and Immunomodulating Effect of the Lectin from Allomyrina dichotoma.

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A new lectin from Allomyrina dichotoma (ADL) was purified by physiological saline extraction, ammonium sulfate fractionation, anion exchange column chromatography on DEAE Sephadex A-50 and gel filtration column chromatography on Sephadex G-200.
Several biochemical properties of this lectin were characterized and the results are as follow :
0.1M fraction of ADL from gel filtration column chromatography showed one band on SDS-PAGE.
A purified lectin agglutinated the erythrocytes of rabbit and human A, B, O, AB.
Agglutinability was relatively stable at basic pH, and was stable at temperature below 40℃. The lectin activity was not affected by some metal ions and chealating agent, EDTA.
The molecular weight of ADL was estimated to be 97,000 dalton by SDS-polyacrylamide gel electrophoresis.
The lectin's immunomodulating effect was measured as cytokine production from peripheral blood mononuclear cells(PBMC) by ELISA(enzyme linked immunosorbent assay).
1×106 cells/㎖ of PBMC were obtained from healthy volunteers and stimulated with ADL for various times(1, 4, 8, 24, 48, 72 and 96 hours). ADL was prepared as 0.3 of optical density. Assay for 5 cytokines (IL-1α, IL-2, IL-6, IFNγ and TNFα) production was measured and the highest cytokine secretion was demonstrated at 24 hours with IFNγ and at 4 hours with TNF-α.