Pentacyclic triterpenoid compounds have various biological effects, such as anti-inflammatory, anti-hyperlipidemia, wound healing and anti-tumor activities. In this study we investigated cytotoxic effects of asiatic acid (AA), a triterpene acid, on human liver cells. AA decreased viability of HepG2 human hepatoma cells in a time- and concentration-dependent manner. Treatment with 40 M AA markedly induced apoptosis in the cells, measured by DNA ladder formation and flow cytometric analysis of hypodiploid DNA stained with propidium iodide and loss of phospholipid asymmetry detected by annexin-V binding. AA also increased intracellular Ca^{2+} level, which was blocked by TMB-8 and dantrolene, intracellular Ca^{2+} release blockers, but not by EGTA, an extracellular Ca^{2+} chelator. Moreover, AA-induced apoptosis was significantly blocked by the co-treatment with TMB-8 and dantrolene, suggesting that intracellular Ca^{2+} release plays an important role in AA-induced apoptosis in HepG2 cells.

[OB-2] [04/20/2001 (Fri) 13:45 - 14:00 / Room 2]

Urushiol Induced Apoptosis and Cell Cycle Arrest in Human Gastric Cancer Cells

Kim SH, Im EO, Yee SB, Jung NC[1], Na CS[1], Kim KW[2], Kim ND

Dept. of Pharmacy, Pusan National University, Pusan, Korea, [2]Dept. of Pharmacy, Seoul National University, Seoul, Korea, [1]Division of Special Purpose Tree, Foresty Research Institute, Suwon, Korea

Urushiols are mixtures of olefinic catechols with a n-C15 or n-C17 alkyl side chain which is isolated from the sap of Korean lacquer tree (Rhus vernicifera Stokes). In the previous study, we evaluated the cytotoxic effect of urushiol on MKN-45 and MKN-28 cell lines. Urushiol mediated DNA fragmentation and nuclear condensation only on MKN-45 cells. In the present study we investigated the modulation of the expression of the apoptosis-related proteins after treatment of urushiol on both MKN-45 and MKN-28 cells. In MKN-45 cells the expression levels of Fas and Fas-ligand proteins were increased after treatment of urushiol. The Bcl-2 protein level was not changed but the Bax protein level was slightly upregulated on MKN-45 cells by the treatment of urushiol. In addition, caspase-3 was activated and PARP protein was cleaved on MKN-45 cells by urushiol treatment. In MKN-28 cells Bcl-2 and Bax protein level were not changed by urushiol treatment. Nevertheless, the cyclin-dependent kinase inhibitors, p21WAF1/CIP1 and p27KIP1 proteins, were upregulated on MKN-28 cell by urushiol. And cyclin-dependent kinase 2 and 4 proteins were decreased. The cyclin-dependent kinase inhibitor protein, a key regulatory protein of the cell cycle, may have contributed to cell cycle arrest in the urushiol-treated MKN-28 cells. These data suggested that urushiol induced apoptosis on MKN-45 cells and mediated cell cycle arrest on MKN-28 cells.

Oral Presentations - Field C

[C1. Biochemistry] [C2. Microbiology] [C3. Cell Biology]

[OC-1] [04/20/2001 (Fri) 14:00 - 14:15 / Room 2]

MODULATION OF HUMAN CYTOCHROME P450 3A4 EXPRESSION BY CERAMIDE, A LIPID SECOND MESSENGER

Chun, YJ, Lee, SH, Yang, SA, and Kim, MY

College of Pharmacy, Chungang University, Seoul 156-756

Cytochrome P450 3A4 enzyme is responsible for the metabolic activation and inactivation of the majority of clinically used drugs in human liver and intestines. Recently it was reported that inflammatory stimuli cause changes in the activities and expression levels of various forms of P450. Here, we have shown the effects of ceramide on cytochrome P450 3A4 expression in human colon carcinoma HT-29 cells. Tumor necrosis factor (TNF)-a, which is known to produce ceramide in cells, blocked the synthesis of P450 3A4. Treatment with synthetic C6-ceramide or bacterial sphingomyelinase (SMase) also strongly suppressed expression of human P450 3A4 in concentration and time-dependent manner. To test the possibilities of cross-talk between inducible nitric oxide synthase (iNOS) and P450 3A4, the effect of nitric oxide on P450 3A4 expression was determined. Interestingly, we found that N^G-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of NOS, was able to protect ceramide-dependent suppression of P450 3A4. In contract, the addition of S-nitroso-N-acetylpenicillamine (SNAP), a NO donor, to HT-29 cells reduced P450 3A4 expression. The addition of iNOS antisense oligonucleotide prevented ceramide-induced iNOS expression, and restored P450 3A4 expression. Our results demonstrate that ceramide is a mediator of P450 3A4 suppression by TNF-a, and increased NO from iNOS induction by ceramide signaling may modulate P450 3A4 expression in cells.

[OC-2] [04/20/2001 (Fri) 14:15 - 14:30 / Room 2]

Silibinin enhances C/EBPα and PPARγ expression and induces differentiation of 3T3-L1 preadipocytes

Chung Su Wol, Kang So Na and Kim Tae Sung

Immunology Laboratory, College of Pharmacy,

The effects of silibinin, an active flavonoid of milk thisfle, on the adipocyte differentiation were studied in a 3T3-L1 adipocyte model in vitro. Silibinin was found to stimulate differentiation of 3T3-L1 cells in a dose-dependent manner. Silibinin also induced the intracellular levels of CCAAT enhancer binding protein(C/EBP)α and peroxisome proliferator activated receptor(PPAR)γ in a dose-dependent manner, as demonstrated by RT-PCR and immunoblots analysis. In the transfection experiments, silibinin induced PPARγ2 promoter activation in 3T3-L1 preadipocytic cells transiently cotransfected with a C/EBPα expression vector. During adipogenesis in culture, silibinin also induced the expression of several genes that are known to turn on during adipocytic differentiation, such as ap2, Adn, IRS-1 and GLUT4. Furthermore, gel shift assays revealed that silibinin decreased NF-κB-DNA binding and enhanced PPRE-DNA binding. Taken together, these results suggest that silibinin enhances adipocyte differentiation though the specific induction of C/EBPα and PPARγ.

[OC-3] [04/20/2001 (Fri) 14:30 - 14:45 / Room 2]

Compound A6792-2 inhibits preferentially the mycelial phase of Candida albicans

Hwang EI, Yun BS, Kim NR, Kang TH, Kim YK and Kim SU°

Antibiotics Research Lab., Korea Research Institute of Bioscience and Biotechnology, P.O.Box 115, Taejon 305-333, Korea

Candida albicans is an oppurtunistic pathogen of humans which shows either yeast-like form or pseudomycelium form in response to different environmental conditions, and the switch from a yeast-like form to a filamentous form often correlates with pathogenicity. Fungal pathogens such as C.