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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

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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

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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.

albicans are of increasing concern because of the rising incidence of immunosuppression brought about by AIDS, diabetes, cancer therapies, organ transplantation, and other conditions.
In the course of our screening for the specific growth inhibitors against the mycelial phase of C. albicans, we have selected a Streptomyces sp. A6792 from soils. Isolation and purification of compound A6792-2 were performed using silica gel column chromatography, ODS column chromatography, preparative silica gel TLC, and Sephadex LH-20 column chromatography. The molecular weight of compound isolated from Streptomyces sp. A6792 was determined as 844. From several spectral analyses, the compound A6792-2 was identified as IKD-8344. This compound exhibited a potent growth inhibitory activity (MIC : 1.56-25 μg/ml) against the mycelial, but not yeast phase of C. albicans (up to 200 μg/
ml).

[OC-4] [04/20/2001 (Fri) 14:45 - 15:00 / Room 2]

Apicidin, a histone deacetylase inhibitor, induces apoptosis and Fas/Fas ligand expression in human acute promyelocytic leukemia cells

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We previously reported that apicidin, a histone deacetylase inhibitor, arrests human cancer cell growth through selective induction of $p21^{WAF1/Cip1}$. In this study, we evaluated the apoptotic potential of apicidin in human acute promyeolcytic leukemia cells HL60. Treatment of HL60 cells with apicidin caused decrease in viable cell number in a dose dependent manner, concomitant with progressive accumulation of hyperacetylation of histone H4. These effects were paralleled by increase in DNA fragmentation, nuclear morphological change, and apoptotic body formation. In addition, apicidin activated caspase-3 through conversion of the proenzyme form of caspase-3 to the catalytically active effector protease, and caused subsequent cleavages of poly(ADP-ribose)polymerase (PARP) and $p21^{WAF1/Cip}$, substrates of the caspase-3. Incubation with z-DEVD-fmk, a caspase-3 inhibitor, almost completely abrogated activation of caspase-3, DNA fragmentaion, cleavage of PARP and $p21^{WAF1/Cip}$ by apicidin, indicating that apicidin-induced apoptosis might be due to the activation of caspase-3. Moreover, these effects were preceded by increase in translocation of Bax into mitochondria, resulting in release of cytochrome c from mitochondria to cytosol and cleavage of procaspase-9. Addition of cycloheximide greatly inhibited apicidin activation of caspase-3 through interfering with cleavage of procaspase-3, suggesting that apoptotic induction by apicidin is dependent on de novo protein synthesis. Consistent with these results, apicidin increased the expression level of both Fas and Fas ligand transiently, which can initiate the apoptotic signalling pathway. Taken together, the results suggest that apicidin induce apoptosis through selective induction of Fas/Fas ligand, resulting in cytochrome c release from mitochondria to cytosol and subsequent activation of caspase-9 and caspase-3.

Oral Presentations - Field D

[D1. Medicinal Chemistry] [D2. Pharmacognosy] [D3. Oriental Medicine] [D4. Analytical Chemistry]

[OD-1] [04/20/2001 (Fri) 13:30 - 13:45 / Room 3]