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Wogonin (5,7-dihydroxy-8-methoxyflavone), isolated from Scutellaria radix, was previously reported to inhibit the expression and activity of cyclooxygenase-2 in lipopolysaccharide stimulated cells of a mouse macrophage cell line, RAW 264.7. Here, in order to find in vivo effects, inhibition by wogonin of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced cyclooxygenase-2 expression and anti-inflammatory activity in vivo were investigated. When applied topically to the dorsal skin of mice, wogonin at doses of 50 ?200 mg/site/treatment (total five treatments in three days) inhibited cyclooxygenase-2 expression and prostaglandin E2 production induced by multiple treatments with TPA. At 200 mg/site/treatment, wogonin caused a 55.3% reduction of prostaglandin E2 production on the dorsal skin compared with an increased production in the TPA-treated control group. The same compound significantly inhibited mouse ear edema induced by TPA in both preventive (58.1% inhibition) as well as curative treatment (31.3% inhibition) schedules at 200 mg/ear/treatment. Inhibition of neutrophil infiltration was also observed. Therefore, wogonin may be beneficial for cyclooxygenase-2-related skin disorders.

[PC1-23] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Effects of Ginkgetin from Ginkgo biloba Leaves on Cyclooxygenases and In Vivo Skin Inflammation

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Ginkgetin, a biflavone from Ginkgo biloba leaves, was previously reported to be a phospholipase A2 inhibitor and this compound showed the potent antiarthritic activity in rat adjuvant-induced arthritis as well as analgesic activity. This investigation was carried out to find effects on cyclooxygenase (COX)-1 and ? including in vivo effect. Ginkgetin (1 ?10 uM) and the biflavonoid mixture (10 ?50 ug/ml), mainly 1:1 mixture of ginkgetin and isoginkgetin, from G. biloba leaves, inhibited production of prostaglandin E2 from lipopolysaccharide-induced RAW 264.7 cells. This inhibition was mediated, at least in part, by down-regulation of COX-2 expression, but not by direct inhibition of COX-1 or COX-2 activity. Down-regulation of COX-2 by ginkgetin was also proved in the dorsal skin of ICR mouse treated by 12-O-tetradecanoylphorbol-13-acetate (TPA). At total doses of 1,000 ug/site on the dorsal skin (15 mm ?15 mm), ginkgetin inhibited prostaglandin E2 production by 65.6% along with marked suppression of COX-2 induction. In addition, ginkgetin and the biflavonoid mixture (100 ?1,000 ug/ear) dose-dependently inhibited skin inflammation of croton oil induced ear edema in mice by topical application. Present study suggests that ginkgetin from G. biloba leaves down-regulates COX-2 induction in vivo and this down-regulating potential is associated with anti-inflammatory activity against skin inflammatory response.

[PC1-24] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Characterization of a gene cluster responsible for catechol catabolism in Pseudomonas cepacia G4

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Pseudomonas cepacia G4 is a soil bacterium that can grow in toluene, cresol or phenol as the sole carbon and energy source. A recombinant plasmid encoding a gene cluster responsible for degradation of the aromatic xenobiotics was isolated from a total DNA library of P. cepacia G4 and designated as pCNU301. The pCNU301 contained tomBCEGFD gene cluster which can encode 6 enzymes catabolizing catechol to acetyl-CoA. In this study, nucleotide sequences of tomFD gene encoding 4-hydroxy-2-

oxovalerate aldolase and 4-oxalocrotonate decarboxylase were determined. The 4-hydroxy-2-oxovalerate aldolase gene (tomF) was consisted of 1047 bases. Amino acid sequence of the tomF gene product exhibited 84% identity with those of 4-hydroxy-2-oxovalerate aldolase from Comamonas testosteroni and P. putida. The 4-oxalocrotonate decarboxylase gene (tomD) was consisted of 687 bases. Amino acid sequence of the tomD gene product exhibited 75% identity with that of 4-oxalocrotonate decarboxylase from P. putida.

[PC1-25] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Activation of $p21^{WAF1/Cip1}$ transcription through Sp1 sites by histone deacetylase inhibitor apicidin: Involvement of protein kinase C

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We previously reported that apicidin, a novel histone deacetylase (HDAC) inhibitor, inhibited the proliferation of tumor cells via induction of $p21^{WAF1/Cip1}$. In this study, we determined the molecular mechanisms by which apicidin induced the $p21^{WAF1/Cip1}$ gene expression in HeLa cells. Apicidin induced $p21^{WAF1/Cip1}$ mRNA independent of the de novo protein synthesis and activated the $p21^{WAF1/Cip1}$ promoter through Sp1-3 site located at -, 82 and -, 77 relative to the transcription start site. Calphostin C, a protein kinase C (PKC) inhibitor, significantly attenuated the activation of $p21^{WAF1/Cip1}$ promoter via Sp1 sites, which was accompanied by a marked suppression of $p21^{WAF1/Cip1}$ mRNA and protein expression induced by apicidin. Consistent with the transcriptional activation of $p21^{WAF1/Cip1}$ promoter by apicidin, apicidin treatment led to the translocation of PKCε from cytosolic to particulate fraction, which was reversed by pretreatment with calphostin C, indicating the involvement of PKC in the transcriptional activation of $p21^{WAF1/Cip1}$ via Sp1 sites by apicidin. However, the PKC-mediated transcriptional activation of $p21^{WAF1/Cip1}$ by apicidin appears to be independent of the histone hyperacetylation, since apicidin-induced histone hyperacetylation was not affected by calphostin C. Furthermore, a PKC activator, PDBu alone induced the transcriptional activation of $p21^{WAF1/Cip1}$ promoter, $p21^{WAF1/Cip1}$ mRNA and protein expression, without induction of the histone hyperacetylation, suggesting that the transcriptional activation of $p21^{WAF1/Cip1}$ by apicidin might have been mediated by a mechanism other than chromatin remodeling through the histone hyperacetylation. Taken together, these results suggest that the PKC signaling pathway plays a pivotal role in the transcriptional activation of the $p21^{WAF1/Cip1}$ gene by apicidin.

[PC1-26] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

2D/MALDI-TOF MS Analysis of Age-dependent Rat Proteome in Rat Liver Mitochondria

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Mitochondria is called power plant of the cell because they product biological energy, ATP, using electron transport system and proton pump. This system is very toxic to living cells because these systems generate the superoxide anion radical and hydroxyl radical, which induce apoptosis via