Cinnamaldehyde induces a decrease in the mitochondrial membrane potential in human leukemia HL-60 cells

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In the previous report, we found that cinnamaldehyde, isolated from the stem bark of Cinnamomum cassia, induced cytotoxicity and apoptosis. These effects were completely prevented by pretreatment with antioxidant N-acetyl-L-cystein (NAC). Cinnamaldehyde activated various caspases, such as caspase-3, caspase-8 and caspase-9 activities. Now we are further investigating the relationship with the mitochondrial membrane potential and the release of cytochrome-c from mitochondria into the cytosol. We measured $\Delta\Psi$ m using the fluorescent probe DiOC6 and monitored it using flow cytometry. Mitochondrial release of cytochrome c was conformed by western blotting. Furthermore, we are undergoing the structure-activity relationship with various cinnamaldehyde derivatives.

[PC1-17] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Antioxidant Effect of Kombucha Extract on Normal Human Diploid Fibroblasts (HDFs)

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Kombucha broth (KB) is a traditionally known remedy in the far east countries. Although many physiological benefits of KB have been reported to date, enough experimental evidences have not been presented yet. Therefore, we attempted to investigate the antioxidant effects on Human Diploid Fibroblasts (HDFs) which was treated with KB extract. Exponentially growing early-passage HDFs were treated with 1 mM Hydrogen Peroxide (H_2O_2) to induce oxidative stress. When the cells get stressed, morphological and biological changes were observed. Following external stress to the cells, incubation with the KB extract for 48hrs was performed. The enzymatic activities of Superoxide Dismutase (SOD), Glutathione Peroxide (GPx) and Catalase (CAT) on H_2O_2-treated cells were significantly higher than those on the non-treated control. However, in the case of H_2O_2 treated HDFs followed by incubation with KB extract the enzymatic activities were sharply reduced in comparison with the only H_2O_2-treated cells. In these data, we draw the following conclusion that KB extract is a possible material to be utilized for the anti-oxidant agent.

[PC1-18] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

EFFECTS OF GENISTEIN ON EXPRESSION OF COX-2 AND ACTIVATION OF ERK 1/2 INDUCED BY PHORBOL ESTER AND TNF-α IN CULTURED HUMAN BREAST EPITHELIAL CELLS

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Genistein has been shown to exert protective effects against chemically induced carcinogenesis in animals as well as malignant transformation in cultured cells, but molecular mechanisms of its chemopreventive or chemoprotective activities remain largely unresolved. In the present study, we have investigated the effects of genistein on induction of cyclooxygenase-2 (COX-2) that plays an important role in the pathophysiology of carcinogenesis as well as in cellular response to inflammatory stimuli. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) or TNF-α caused dose- and time-dependent increases in COX-2 expression and prostaglandin E_2 (PGE_2) production in MCF10A cells, which was inhibited by genistein pretreatment. Inhibition of PGE_2 production by genistein appeared to be attributable to its suppression of both catalytic

activity and expression of COX-2. There are multiple lines of evidence supporting that the induction of COX-2 is regulated by the eukaryotic transcription factor NF-κB. TPA stimulated both NF-κB DNA-protein binding and COX-2 promotor activity. However, genistein did not inhibited TPA- or TNF-α-induced NF-κB DNA-protein binding, but suppressed the transcriptional activity of NF-κB induced by TPA. Immunofluorescence staining also demonstrated that increased nuclear translocation of the active NF-κB p65 subunit was not abolished by genistein. Genistein treatment attenuated TPA- or TNF-α-induced activation of ERK1/2. Above findings, taken together, suggest that genistein inhibits COX-2 expression and PGE_2 production in MCF10A cells by acting at the transcription initiation complex via a tyrosine kinase- or ERK-dependent pathway.

[PC1-19] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Differential inhibitory effects of sophoricoside and its analogs on COX isozymes

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Cyclooxygenase (COX), COX-1 and 2 catalyzes the conversion of arachidonic acid to prostaglandin H2, rate-limiting step of prostanoid biosynthesis. COX-1 is constitutive expressed in most tissues under physiological conditions, whereas COX-2 is induced by some cytokines, mitogens, and endotoxin presumably in pathological conditions, such as inflammation. In this study, inhibitory effects of sophoricoside and its analogs on COX-1 and COX-2 activities has been evaluated. Microsomal fraction of bovine seminal vesicles was used as the COX-1 source, and lysate of LPS-stimulated murine macrophages Raw 264.7 as the COX-2 source. COX activity was measured by chemiluminescence emitted for 30 sec in the presence of arachidonic acid and luminol. Inhibitory potencies of the compounds on COX-2 were in the order of orobol (IC50=0.8 uM) > genistein (IC50=7.7 uM) > genistin (IC50=8.7 uM) > sophoricoside (IC50=9.3 uM). Selective COX-2 inhibitor NS-398 showed an IC50 value of 0.23 uM on COX-2 activity and 48.9 uM on COX-1 activity. Orobol and genistein exhibited IC50 values of 7.9 uM and 72.6 uM on COX-1 activity, respectively, and sophoricoside and genistin of >100 uM. All of the compounds did not affect the expression and synthesis of COX-2 in the LPS-stimulated murine macrophages Raw 264.7, which was analyzed by RT-PCR. These pharmacological findings will be helpful to analyze protective mechanisms of flavonoid compounds against inflammatory conditions.

[PC1-20] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Spiciformisin-b and Moncyclicsqualene Induce Differentiation and Apoptosis of Human Leukemic cell HL-60

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From the leaves of Ligularia Fischery var. spiciformis, a new terpenoids named spiciformisin-b and moncyclicsqualene were isolated. These compounds have a antitumor activity by induction of cell differentiation and apoptosis in HL-60. Spiciformisin-b and moncyclicsqualene were found to be a potent inducer of differentiation toward granulocyte and monocyte/
macrophage lineages. The effect of differentiation has been detected by esterase activity, phagocytosis, NBT reduction and CD14, CD66b surface antigen. Moreover, they showed apoptosis inducing effect at a concentration of 40㎍/㎖ These apoptotic features were identified by increasing of hypodiploid nuclei and nucleosomal ladder. These results suggest that spiciformisin-b and moncyclicsqualene induce differentiation and apoptosis in HL-60 cells.

[PC1-21] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]