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Ginsenoside Rc which was extracted from *Panax Ginseng* C. A. Meyer was purified. The saponins of Ginsenoside Rc group are known to have many pharmacological effects related to anticancer activity. So, the compound was treated at SK-MEL-28 human skin cancer cell line to define apoptosis. And then, MTT assay, cell cycle analysis, Terminal Deoxyribonucleotidyl Transferase-Mediated dUTP Nick End Labeling (TUNEL) assay, and Fas expression were performed for the study.
MTT assay was performd to determine cytotoxicity of Ginsenoside Rc at various times and concentrations. Cell cycle analysis by flow cytometer showed that the cell cycle arrested at S phase. And we examined that the compound induced apoptosis of the cell by TUNEL assay to characterize apoptosis. Fas expression depended on time and concentration evidenced that cell death was induced by interaction of Fas and Fas ligand (CD95).
These data suggested that Ginsenoside Rc induced apoptosis in SK-MEL-28 Human Melanoma Cell Line.

[PC1-27] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Effects of Tanshinone I Isolated from Salvia miltiorrhiza Bunge on Arachidonic Acid Metabolism and In Vivo Inflammatory Responses

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We have evaluated 300 plant extracts for their inhibitory activity of PGD2 production from cytokine-induced mouse bone marrow-derived mast cells in vitro. From this screening procedure the methanol extract of Salvia miltiorrhiza was found to inhibit PGD2 production and the ethylacetate subfraction gave the strongest inhibition among 5 subfractions tested. From this ethylacetate subfraction, an activity-guided isolation finally gave tanshinone I as an active principle. This investigation deals with the effects of tanshinone I on AA metabolism from lipopolysaccharide (LPS)-induced RAW 264.7 cells and in vivo anti-inflammatory activity. Tanshinone I inhibited PGE2 formation from LPS-induced RAW macrophages (IC50 = 38 uM). However, this compound did not affect COX-2 activity or COX-2 expression. Tanshinone I was found to be an inhibitor of type IIA human recombinant sPLA2 (IC50 = 11 uM) and rabbit recombinant cPLA2 (IC50 = 82 uM). In addition, tanshinone I showed in vivo anti-inflammatory activity in rat carrageenan-induced paw edema and adjuvant-induced arthritis.

[PC1-28] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

In Vivo Protection of the Flowers of Prunus persica Extract from Solar Ultraviolet-Induced Skin Damage

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The flowers of Prunus persica Batsch have been used for skin disorders in East Asia from ancient time. In this investigation, the ethanol extract from this plant materials was prepared and evaluated for the protective effects on solar ultraviolet (UV)-induced skin damages including UVB-induced erythema in guinea-pigs, ear edema in ICR mice and skin carcinoma in SKH-1 mice. The P. persica extract clearly inhibited UVB-induced erythema formation dose-dependently when topically applied (IC50 = 0.5 mg/cm2). And it also inhibited UVB-induced ear edema (49% inhibition at 3.0 mg/ear). In addition, the extract greatly reduced UVB-induced skin tumor formation (42% reduction of tumor incidence and 71% reduction of total number of tumors in 28 weeks) at the initial dose of 5.0 mg/cm2/day and the maintenance dose of 2.5 mg/cm2/day). From the extract, four kaempferol glycoside derivatives were successfully isolated and their contents were measured with HPLC. Among the derivatives, the content of multiflorin B was highest and this compound inhibited UVB-induced erythema formation, indicating that multiflorin B is one of the active principles of the extract. All these results suggest that the flowers of P. persica extract may be useful for protection against UV-induced skin damage when topically applied.

[PC1-29] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Cloning of nitric oxide synthase from Staphylococcus aureus

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Recently, we have reported the existence of bacterial nitric oxide synthase (NOS) in the prokaryotic cell, *Staphylococcus aureus* (*S. aureus*). The bacterial NOS in *S. aureus* requires the existence of cofactors (FMN, FAD, NADPH, BH_4 and calcium/calmodulin) and is induced by methanol. We compared the cofactor binding sites of murine iNOS with total genomic sequence of *S. aureus* and found seven matching sequences. Using homologous primers, we amplified 0.85kb DNA fragment from genomic DNA library of *S. aureus*. Screening of genomic DNA library produced twelve positive clones. Four DNA fragments (2, 4.5, and two 6 kb) were selected by Southern blot analysis and ligated into mammalian expression vector, pcDNA3.1.

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

Detection of Proteins in Sodium Dodecyl Sulfate -Polyacrylamide Gel Electrophoresis Using a Counter Ion Dye Staining Technique with Coomassie Brilliant Blue R / Neutral Red

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Coomasie Brilliant Blue R (CBBR) staining method has been used the most widely in the detection of proteins in Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). However, it requires long staining time and can't be visualized in course of staining. Here we describe fast, sensitive and simple staining method that can be visualized in the course of staining employing a counter ion dye staining technique. Acidic dye, CBBR(0.005%) was mixed up with basic dye, Neutral Red (NR) (0.0005%). After electrophoretic separation in SDS-PAGE, gels were fixed with 40%MeOH/7%HAc for 30min. Then, gels were stained in 0.005%CBBR-0.0005%NR, in 30% MeOH/7%HAc staining solution for 30min-1hr. It was preferable to wash gels for 10 minutes after