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Comet assay has been applied to the detection of DNA damage due to environmental toxic materials. In particular, this assay is a novel method to assess DNA single-strand breaks. Four weeks old ICR male mice were irradiated with 3.5 Gy of γ-ray five days after oral administration of antioxidants such as ascorbic acid and cysteine and were sacrificed 3 days later to prepare splenocytes. The tail moment of DNA single-strand breaks in the splenocytes was evaluated by the comet assay. The treatment of the antioxidants reduced the tail moment in the comets compared with that of the irradiated control group. This result indicates that antioxidants like ascorbic acid and cysteine have radioprotective effects on the splenocyte DNA when assessed by the comet assay.

[PA3-9] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Effects of Leucocyanidin on Acetaminophen-induced Hepatotoxicity nd the Mechanism

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To examine the protective effect of Leucocyanidins (LC) on the liver injury, mice were pretreated orally with LC (50, 100 and 150mg/kg/day) for 7days and then injected intraperitoneally with 400mg/kr AA. LC pretreatment (100 and 150mg/kg/day) markedly decreased the incidence and severity of hepatic necrosis in mice given AA. In mice pretreated with LC, the mortality dose-dependently decreased compared to that of mice given AA. These results indicate that LC pretreatment has a protective effect on AA-induced hepatotoxicity.
After treatment with LC (50 and 100mg/kg/day, p.o.) for 7days, the enzyme activities of cytochrome P450 monooxygenases, UDP-glucoronyl transferase (UDP-GT), phenol sulfotransferase (PST), glutathione S-transferase (GST) were measured in SD-rat livers. Also, antioxidant enzyme activities, e.g., superoxide dismutase, catalase and glutathione peroxidase were measured after LC treatment. Treatment with 100mg/kg/day LC markedly decreased the activities of P4501A1, P4501A2, P4502B1, P4503A4, except P4502E1. In contrast, the activities of GST and PST were significantly increased in rats treated with 100mg/kg/day LC. The antioxidant enzyme activities were not decreases the activities of certain P450 isozymes, which are responsible for bioactivation of AA (e.g., P4501A2 and P4503A4) and elevates the activities of PST and GST, which are related to the elimination of AA. Moreover, the protective effects against AA toxicity by LC pretreatment may be related to these regulations of LC on xenobiotic metabolizing enzymes.

[PA3-10] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Polymethoxyflavonoids from Vitex rotundifolia inhibit proliferation by inducing apoptosis in human myeloid leukemia cells

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Three polymethoxyflavonoids from the fruit of Vitex rotundifolia, namely 2',3',5-trihydroxy-3,6,7-trimethoxyflavone (Vx-1), vitexicarpin (Vx-5) and artemetin (Vx-6), were tested for their antiproliferative activity in human myeloid leukemia HL-60 cells. They showed a dose-dependent decrease in the growth of HL-60 cells. The concentrations required for 50% inhibition of the growth (IC50) after 96 h were 4.03 ?M, 0.12 ?M and 30.98 ?M for Vx-1, Vx-5 and Vx-6, respectively.

Treatment of HL-60 cells with the flavonoids induced morphological changes that are characteristic of apoptosis. We judged the induction of apoptosis by the detection of DNA fragmentation in agarose gel electrophoresis and the degree of apoptosis was quantified by a double-antibody sandwich ELISA and by flow cytometric analysis. The C-3 hydroxyl and C-8 methoxyl groups were found not to be essential for the activity, but the C-3' methoxyl instead of hydroxyl group lowered the antiproliferative and apoptosis inducing activity. These results suggest that the polymethoxyflavonoids isolated from V. rotundifolia may be used as potential chemopreventive and chemotherapeutic agents.

[PA3-11] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Screening of natural product inhibitors on the UVB phototoxicity of Chlorpromazine

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15 natural products known to contain antiinflammatory effect were screened whether they have phototoxicity inhibitory effect or not by two methods - RBC photohemolysis test and yeast growth inhibition test using Candida albicans. Samples were obtained by the process of 80 % methanol extraction and then concentration under vaccum. And we made these concentration powder with freeze-dryer at -50~-60 ℃. In RBC photohemolysis method, effects of the test samples on RBCs were monitored with a spectrophotometer by the method of Kahan et al. And in the second method, we dissolved the samples in distilled water(1mg/ml)and injected 50 μl into paper disks and paper disks absorbed 0.6 mg chlorpromazine(CPZ) in advance respectively. Controls were absorbed only CPZ. Diluted Candida albicans suspension was seeded on the Sabouraud's dextrose agar plate, and then the paper disks were located on the plates. The plates were exposed to 2.0 J/cm2 of UVB(312 nm), and futher incubated at 27 ℃ for 24 hr. The diameters of inhibition zones formed around the disks were measured.

[PA3-12] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

The Antiplatelet Mechanism of (-)-Epigallocatechin Gallate: Effect on extracellular calcium mediated aggregation

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We previously reported the antithrombotic and antiplatelet activities of green tea catechins (GTC) and (-)-epigallocatechin gallate (EGCG), a major compound of GTC.
EGCG inhibited the aggregation of platelets in vitro and ex vivo, and prevented pulmonary thrombosis in vivo. EGCG inhibited the GPIIb/IIIa-fibrinogen binding, IP3 formation, ATP release, but elevated the cAMP level.
In the present study, the effects of EGCG on extracellular calcium mediated aggregation induced by thrombin, collagen and A23187 were examined.
In the presence of EGTA, human washed platelet (WP) aggregation was suppressed in response to thrombin, collagen and A23187, respectively. And the platelet aggregation induced by addition of CaCl2 was inhibited by EGCG in a concentration-dependent manner. A23187 can penetrate membranes and directly mobilize Ca2+ from intracellular stores, thereby increasing the cytosolic Ca2+ concentration. To investigate Ca2+ influx and release, the aggregation was induced in the presence of 1 mM CaCl2, Ca2+-free medium and 0.5 mM EGTA-treated WP was also tested. The Ca2+-free WP was incubated in 37℃ for 3 minutes and then induced by 1μM A23187. At the same time, the prepared Ca2+-free WP was added with 1 mM CaCl2 and incubated for 30 minutes at room temperature. The 0.5 mM EGTA-treated WP was prepared by addition of 0.5 mM EGTA in