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 ㎕ 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

Ca2+-free WP and the aggregation was examined. The aggregation was recovered by the addition of CaCl2 at the concentration of 1mM by 70-80%, whereas it was inhibited by EGCG in a concentration-dependent manner.
These results suggest that the influx of extracellular calcium is important in the platelet aggregation and EGCG inhibit the calcium influx from the medium.

[PA3-13] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Regulation of Caspase Activation and cis-diamminedichloroplatinum(II)-induced cell death by KC-1

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Cisplatin (cis-diamminedichloroplatinum, CDDP) is a widely used antineoplastic agent, cisplatin may cause acute renal failure after even a single dose. The underlying mechanism of this nephrotoxicity is still not well known. LLC-PK1 cells express many characteristics of renal proximal tubule epithelia. We report here the use of this cell line to investigate the regulation of caspase activation by KC-1 and the possible mechanisms of alleviative effect of CDDP-induced renal toxicity by KC-1 cisplatin. First, The time- and dose dependency of cisplatin-induced cytotoxicity were established by exposing LLC-PK1 cells to different concentration (0.1 to 100 uM) of cisplatin from 4 to 48 hours. As a result, the cell viability of the 48 Hr-exposured cell has been shifted from 69.5 ± 2.68 (%) at 10 uM to 9.5 ± 1.01 (%) at 50 uM. Second, the protective effect of KC-1 against cisplatin-induced cytotoxicity was studied. The influence of KC-1 was determined by measuring the cell viability. The data showed that the IC50 of the 48 hrs exposured cell has been shifted from 15 uM in an CDDP single treatment to 30 uM in an KC-1 with an range of 50-100 uM. Third, A family of intracellular cysteine proteases, the caspases, is often activated and plays an important role in the dismantling of cell structures during apoptosis initiated by both the external and internal pathways. caspase-3, previously called CPP32/Yama/Apopain, is an ICE-like protein which could be detected in high rate during an apoptosis, as the result of an overexpression. Recently, we are trying to demonstrate an order of the pathway by providing evidence of the regulation of caspase activity and cisplatin-induced cell death pathway by KC-1.

[PA3-14] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Effects of Phellinus linteus extracts on immune function in normal and cyclophosphamide-treated mice.

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The purpose of this research was to investigate immunnomodulating effects of Phellius linteus hot water extract(PL-W) and methanol extract(PL-M) in normal and cyclophosphamide(CY)-treated mice. PL-M or PL-W was adminstered p.o. single(400, 800, 1600 mg/kg) or once a day for 5 days in normal and CY-treated mice, and then splenic IgM plaque forming cells(PFC) against SRBC was assayed. IgM PFC against SRBC was significantly and dose-dependently increased as compared with normal group. Mouse splenocytes was incubated in the presence of various concentration of PL-W(0.5, 1.0, 2.5, 5.0, 7.5 mg/ml) and PL-M (0.1, 0.5, 1.0, 2.5, 5.0 mg/ml) and after 48hrs, splenocyte proliferation(SP) was assessed in vitro by MTT assay. PL-W and PL-M increased significantly and dose-dependently the proliferation of normal mouse splenocytes. PL-M showed higher activity than PL-W. We also examined the effect of Phellius linteus extract on the mitogen (Con A, LPS)-induced splenocyte proliferation. PL-W and PL-M inhibited CY-induced suppression of SP against mitogen. These results suggest that Phellius linteus extract has immunostimulative