PCBs, fraction II :Planar aromatic compounds-PAHs, PCDDs and PCDFs, so-called "TCDD-equivalents") obtained from the fly ash is screened for their estrogenicity and antiestrogenicity in the E-screen assay.

[PA3-20] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Induction of Apoptosis by A Novel Intestinal Metabolite of Ginseng Saponin via Cytochrome c Mediated Activation of Caspase-3 Protease

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Ginseng saponins exert various important pharmacological effects with regard to the control of many diseases including cancer. The novel intestinal bacterial metabolites of ginseng protopanaxadiol saponins have been recently found and isolated after the oral administration of ginseng extract in human and rats. 20-O-(?-D-Glucopyranosyl)-20(S)-protopanaxadiol (IH-901) formed from ginsenosides Rb1, Rb2 and Rc is of particular interest in cancer chemoprevention and treatment. We investigated the effects of IH-901 on human myeloid leukemia cell line HL-60, in terms of inhibition of proliferation and induction of apoptosis. IH-901 showed a significant cytotoxic activity in HL-60 cells (IC50 =24.3 ?M) following a 96 hr incubation. Treatment of HL-60 cells with IH-901 resulted in the formation of internucleosomal DNA fragments. The dose- and time-dependent induction of apoptosis by IH-901 was demonstrated in the sandwich enzyme immunoassay and the results were confirmed by flow cytometric analysis. Morphological examination of IH-901 treated samples showed cells with chromatin condensation, cell shrinkage and nuclear fragmentation, which are typical characteristics of apoptotic cells. The treatment of HL-60 cells with IH-901 caused activation of caspase-3 protease and subsequent proteolytic cleavage of poly(ADP-ribose) polymerase. IH-901 did not affect the expression of antiapoptotic protein Bcl-2 but caused a release of mitochondrial cytochrome c into cytosol. In conclusion, our results demonstrate that IH-901 dramatically suppresses HL-60 cell growth by inducing programmed cell death through activation of caspase-3 protease, which occurs via mitochondiral cytochrome c release independently of Bcl-2 modulation. These results may provide a pivotal mechanism for the use of IH-901 in the prevention and treatment of leukemia.

[PA4-1] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Excretion of optical fenfluramine in the rat at various dosages.

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Fenfluramine, a substituted amphetamine derivative which lacks the psychostimulant effect of amphetamine, is abused as diet pill in Korea because it is freely marketed in China. Fenfluramine is administered orally as the racemic mixture, but it's optical isomers have different actions ; d-Fenfluramine is an anoretic agent, while l-isomer is a neuroleptic agent. An anorectic effect of racemic fenfluramine is due to its d-isomer and it's N-dealkylated metabolite d-norfenfluramine. The metabolism and excretion of fenfluramine isomers were studied in the rat following oral administration of 5, 25 and 40mg/kg of racemic fenfluramine. The enantiomeric separation of fenfluramine was performed on achiral column by gas chromatography using (S)-N-(trichloroacetyl)-L-propyl chloride (TFP-Cl) as a derivatizing agent. Urinary recoveries of l- and d-fenfluramine in urine specimens collected during first 24hr after oral dosing of racemic fenfluramine in rat were 0.72-2.72% & 1.30-5.58% and 4.20-8.17% & 11.53-20.01% in 5mg/kg and 40mg/kg dose respectively. The comparison in the levels of isomers showed that d-fenfluramine were higher than l-form, while d-norfenfluramine were lower than l-form in all doses. The metabolite to parent drug ratio declined on dosage. This indicates that high dose of femfluramine result in transient

saturation of the first-pass metabolism together with concomitant saturation of hepatic clearance in the metabolism of fenfluramine.

[PA4-2] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Simple Screening Method of Endocrine Disruptors using spot-test procedure of yeast-based steroid hormone receptor gene transcription assay

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Simple Screening Method of Endocrine Disruptors using Spot-test procedure of Yeast-based Steroid Hormone Receptor gene transcription assay

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A yeast-based steroid hormone receptor gene trascription assay was previously developed for the evaluation of chemicals with endocrine modulating activity by McDonnell's group at Duke University Medical Center, USA. The yeast transformants used in this assay contain the human estrogen, androgen or progesteron receptor along with the appropriate steroid responsive elements upstream of the β-galactosidase reporter gene. The original procedure of the assay comprised the following step: i) Dilution of early mid-log phase culture to an OD600 of 0.03 in selective medium plus CuSO4 to induce receptor production ii) Addition of either steroid or test chemical, followed by overnight incubation with shaking iii) Dilution to OD600 of 0.25 and aliquotes of 100㎕ added to 96-well microtiter plate iv) Addition of equal volume of assay buffer containing 2-nitrophenol-β-D-galactosidase(ONPG) as a substrate for β-galactosidase v) Measurement the change in concentration of orthonitrophenol using a microtiter plate reader. We here report a simple spot-test procedure using X-gal as a substrate for β-galactosidase instead of ONPG. Production and induction of β-galactosidase can be evidenced on plates containg X-gal which released a colored dye when hydrolyzed by β-galactosidase. Effect of the variation of the medium components and oxalyticase application on the response in this spot assay will also be discussed.

[PA4-3] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Stability Study on DK-35C, a Carbapenem Antibiotics by HPLC : Effects on pH and Time Changes

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Impurity profile study of DK-35C (a carbapenem antibiotics) was conducted by two different methods of HPLC/UV. In Experiment I, Bondapak C18 column and 0.01 M ammonium phosphated buffer with 0.05% triethylamine (pH 6.5)/methanol (85/15, v/v) as mobile phase were used. Parent and its related impurity peaks were monitored for 6 days. In Experiment II, Lichrosorb RP18 column and sodium phosphate buffer (pH 6.5)/methanol (7/3, v/v) as mobile phase were used. Peak areas of DK-35C and its impurity was measured at several different pH values. The result from Experiment I is that DK-35C parent (11.9 min) and four impurity peaks (3.3, 5.8, 10.1, and 19.1 min) were observed and methanol solution of DK-35C showed a rapid degradation after 24 hrs. The maxium wavelength of DK-35C absorption was observed at 300 nm. The results from Experiment II showed