

tamoxifen, it did not affect the effect of tamoxifen. 3) The cell cycle analysis results showed that either all trans retinoic acid or 9-cis-retinoic acid treatment showed increase in G2-M phase and When either all trans retinoic acid or 9-cis-retinoic acid treatment in the presence of estrogen, it did not affect the effect of estrogen. When either all trans retinoic acid or 9-cis-retinoic acid treatment in the presence of tamoxifen, it did not affect the effect of tamoxifen. 4) The mRNA of cycline D1 was increased by either all trans retinoic acid or 9-cis-retinoic acid treatment both in the phenol red + and - medium.

[PA4-13] [ 04/18/2002 (Thr) 14:00 - 17:00 / Hall E ]

Protective effects of paeonol on cultured rat hepatocytes exposed to chemical toxicants.

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Br-A23187 (Ca<sup>2+</sup> inophore) and t-BuOOH (oxidative stressor) are frequently used as models of cell killing as Br-A23187 and t-BuOOH induce both necrotic and apoptotic cell death, respectively. The aim of this study was to evaluate the protective effects of paeonol isolated from Paeonia Moutan on cultured rat hepatocytes exposed to Br-A23187 and t-BuOOH. Cell killing was assessed by propidium iodide fluorometry. Br-A23187 and t-BuOOH caused dose-dependent cell killing. Br-A23187 and t-BuOOH-induced cell killings of hepatocytes were decreased in the presence of paeonol (20, 50, 100  $\mu$ M). On the other hand, Paeonol decreased intracellular [Ca<sup>2+</sup>] level of hepatocytes in a dose-dependent manner. Therefore, the present results indicate that paeonol has protective effects against Br-A2187 and t-BuOOH-induced hepatocytotoxicity in rat, indicating paeonol decreases intracellular [Ca<sup>2+</sup>] level of hepatocytes.

[PA4-14] [ 04/18/2002 (Thr) 14:00 - 17:00 / Hall E ]

Determination of 5 $\alpha$ -Reductase Activity in Glial Cells and Rat Tissues by Gas Chromatography/Mass Selective Detector

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The steroid enzyme 5 $\alpha$ -reductase (5AR) catalyzes the conversion of testosterone to dihydrotestosterone. Generally, 5AR activity was measured from <sup>3</sup>H- or <sup>14</sup>C- labelled testosterone as substrate by TLC or HPLC separation of its product. Radiolabelled substrate is expensive and requires the caution in its treatment. TLC and HPLC separation for the identification of the enzyme product is also the additional step. In our study, we tested to develop the new method for measurement of 5AR activity by GC/MSD without using a radiolabelled substrate. Testosterone as substrate was used for 5AR assay in brain homogenate and this reaction was started by the addition of NADPH (0.5 mM) and terminated by the addition of cold ethyl acetate. The organic layer after extraction was evaporated and the residue was derivatized with silylating agent before injection to GC/MSD. Retention times of testosterone and dihydrotestosterone were 7.2 and 6.8 min, respectively. Calibration curve of dihydrotestosterone showed good linearity ( $r=0.9997$ ). When compared specificity of substrate of dihydrotestosterone with androstenedione, specificity to these substrates were similar ( $1.288 \pm 0.096$  nmol/hr/mg protein for androstenedione vs  $1.160 \pm 0.087$  nmol/hr/mg protein for dihydrotestosterone). Optimal incubation time and substrate concentration were decided to be 30 min and 0.1 mM, respectively. This assay was applied to measure 5AR activity in glial cells and the frontal cortex of rat brain. 5AR activity in male rat brain was about 13-fold higher compared to that in female. This method is suitable for measurement of 5AR activity in cells or tissues.

[PA4-15] [ 04/18/2002 (Thr) 14:00 - 17:00 / Hall E ]

Estimation of Methamphetamine Consumption History by the Sectional Analysis of Hair