

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 (Ca²⁺ 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 μ M). On the other hand, Paeonol decreased intracellular [Ca²⁺] 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 [Ca²⁺] level of hepatocytes.

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

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

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The steroid enzyme 5 α -reductase (5AR) catalyzes the conversion of testosterone to dihydrotestosterone. Generally, 5AR activity was measured from ³H- or ¹⁴C- 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 ± 0.096 nmol/hr/mg protein for androstenedione vs 1.160 ± 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

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Recently hair analysis has been taken great interest because it provides a wider window of drug detection. Once drug incorporated into the hair, it moves along hair shaft at a rate of approximately 1cm/month as the average rate of hair growth.

In this study, the relationship between the distribution of methamphetamine (MA) in hair and drug history was investigated. The scalp hair samples of five MA abusers (6~12cm in length) were obtained for sectional analysis. Hair strand was cut into 2 or 3cm sections from the root side, and drug concentrations of each segment were evaluated by GC/MS. For quantitative determinations, the following ions were used: m/z 140 (AM), 144 (AM-d₅), 154 (MA), and 158 (MA-d₅).

The concentrations of MA and AM in hair segment were exhibited variable patterns according to their drug consumption histories. In case 4, MA consumption in the last 2 months could be proved because the last-grown segment (0~2) was positive and the previously grown segments (2~4 and 4~7) were negative. These results suggest that sectional analysis of hair is useful in determining past drug histories in the field of forensic science. However, it also has to be taken into account that the growth rate of hair can vary between 0.8 and 1.4 cm/month and that the telogen partition of the hair can increase to 20%.

[PA4-16] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Metallothionein-III Inhibits hydroxyl radical-induced DNA damage and scavenges superoxide radicals

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Metallothionein (MT)-III is a member of a brain specific MT family, in contrast to MT-I and MT-II that are found in most tissues and are implicated in metal ion homeostasis and antioxidant. To investigate the defensive role of MT-III in terms of hydroxyl radical-induced DNA damage, we used purified human MT-III. DNA damage was detected by single strand breaks of plasmid DNA and deoxyribose degradation. In this study, we show that MT-III is able to protect against the DNA damage induced by ferric ion-nitrosotriacetic acid and H₂O₂, and that this protective effect is inhibited by the alkylation of the sulfhydryl groups of MT-III by treatment with EDTA and N-ethylmaleimide. MT-III was also able to efficiently remove the superoxide anion, which was generated from the xanthine/xanthine oxidase system. These results strongly suggest that MT-III is involved in the protection of reactive oxygen species-induced DNA damage, probably via direct interaction with reactive oxygen species, and that MT-III acts as a neuroprotective agent.

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

Role of Increases of Glutamine Synthetase in Primary Culture of Mixed Glial Cells (MGC) and Purified Astrocytes (AST) on Methylmercury Toxicity

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Methylmercury (MeHg) is a highly neurotoxic compound producing neuronal death that is partially mediated by glutamate. Although MeHg produces the neuronal death and malfunction, MeHg toxicity in glial cells is not clear. Glutamine synthetase (GS), known as a glial-specific enzyme, catalyzes the synthesis of glutamine from glutamate and ammonia and is associated with ischemic injury and several neurological diseases. *Dysregulation of glutamate, an excitatory neurotransmitter, may cause excitotoxicity.* Objective of this experiment is to investigate whether MeHg exposure has adverse effects on GS and whether glutamate plays a role in MeHg-induced toxicity of the MGC and AST. To MGC and AST cultured from the cerebral cortex of one day-old rats, MeHg (0.5 and 10 μM) was exposed for 6 days from 5 days in vitro. MeHg exposure produced dose-dependent increases of GS activity in MGC and AST. Cell viability, total cell