

microsomal activity of p-nitrophenol hydroxylase was dose-dependently suppressed. In addition, the activities of benzyloxyresorufin- and pentoxyresorufin-O-dealkylases were dose-dependently induced by the treatment with DAS. The Western immunoblotting also showed the suppression of P450 2E1 and the induction of P450 2B1/2 and P450 3A1/2 proteins. To investigate a possible role of metabolic activation by P450 enzymes in thioacetamide-induced hepatotoxicity, rats were pre-treated with 400 mg/kg of DAS for 3 days, followed by a single intraperitoneal treatment with 100 and 200 mg/kg of thioacetamide in saline for 24 hr. The activities of serum alanine aminotransferase and aspartate aminotransferase greatly increased by thioacetamide were recovered in DAS-pretreated animals. Taken together, our present results indicated that 1,8-cineole and DAS might be useful P450 modulators in investigating the possible role of metabolic activation in chemical-induced hepatotoxicity and immunotoxicity. (This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ2-PG3-21605-0002).

[PA4-43] [ 04/17/2003 (Thr) 14:00 – 17:00 / Hall P ]

### Development and Validation of the Custom Human cDNA Microarray (KISTCHIP-400) for Monitoring Expression of Genes Involved in Hormone Disruption

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Transcript profiling is a particularly valuable tool in the field of steroid receptor biology, as these receptors are ligand-activated transcription factors and therefore exert their initial effects through altering gene expression in responsive cells. Also, an increased awareness of endocrine disrupting chemicals (EDCs) and their potential to affect wildlife and humans has produced a demand for practical screening methods to identify endocrine activity. Here we developed an in-house cDNA microarray, named KISTCHIP-400, with 401 clones, hormone related genes, factors, and ESTs, based on public database and research papers. These clones contained estrogen, androgen, thyroid hormone & receptors, sex hormone signal transduction & regulation, c-fos, c-myc, ps2 gene, metabolism related genes etc. And to validate the KISTCHIP-400, we investigated gene expression profiles with reference hormones,  $10^{-8}$  M 17beta-estradiol,  $10^{-7}$  M testosterone,  $10^{-7}$  M progesterone, and thyroxin in MCF-7 cell line. Although it is in first step of validation, low doses and combinations of EDCs need to be tested. Our preliminary results that indicate the developed microarray may be a useful laboratory tool for screening EDCs and elucidating endocrine disrupting mechanism.

[PA4-44] [ 04/17/2003 (Thr) 14:00 – 17:00 / Hall P ]

### Identification of Differentially Expressed Genes by Methylmercury in Neuroblastoma cell line using suppression subtractive hybridization (SSH) and cDNA Microarray

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Methylmercury (MeHg), one of the heavy metal compounds, can cause severe damage to the central nervous system in humans. Many reports have shown that MeHg is poisonous to human body through contaminated foods and has released into the environment. Despite many studies

on the pathogenesis of MeHg-induced central neuropathy, no useful mechanism of toxicity has been established so far. In this study, two methods, cDNA Microarray and SSH, were performed to assess the expression profile against MeHg and to identify differentially expressed genes by MeHg in neuroblastoma cell line. TwinChip Human-8K (Digital Genomics) was used with total RNA from SH-SY5Y (human neuroblastoma cell line) treated with solvent (DMSO) and 6.25  $\mu$ M ( $IC_{50}$ ) MeHg. And we performed forward and reverse SSH method on mRNA derived from SH-SY5Y treated with DMSO and MeHg (6.25  $\mu$ M). Differentially expressed cDNA clones were sequenced and were screened by dot blot and ribonuclease protection assay to confirm that individual clones indeed represent differentially expressed genes. These sequences were identified by BLAST homology search to known genes or expressed sequence tags (ESTs). Analysis of these sequences may provide an insight into the biological effects of MeHg in the pathogenesis of neurodegenerative disease and a possibility to develop more efficient and exact monitoring system of heavy metals as common environmental pollutants.

Poster Presentations – Field B1. Physiology

[PB1-1] [ 04/18/2003 (Fri) 09:30 – 12:30 / Hall P ]

**Effects of protein kinase inhibitors on mellitin-induced histamine release in RBL 2H3 mast cells**

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It has been previously reported that silica dose-dependently caused the increase of histamine release and arachidonic acid release in RBL 2H3 cells. In this study, to investigate role of arachidonic acid in inflammatory response including histamine release and reactive oxygen species (ROS) generation, we observed effects of mellitin on histamine release and ROS generation in RBL 2H3 cells. Mellitin, an endogenous phospholipase A2 activator, dose-dependently increased both histamine release and arachidonic acid release, whereas decreased the generation of ROS and peroxynitrite. Mellitin-induced histamine release was significantly inhibited by MAFP (cPLA2 inhibitor, 10  $\mu$ M) and bromoenol lactone (iPLA2 inhibitor, 10  $\mu$ M), but not by OPC and mepacrine (secretory PLA2 inhibitor). Arachidonic acid release induced by mellitin was augmented by histamine receptor antagonists (pyrilamine and cimetidine), which indicate that histamine may be involved in arachidonic acid release via negative feedback mechanism. On the other hand, mellitin-induced histamine release was significantly inhibited by DHC (tyrosine kinase inhibitor, 10  $\mu$ M) and worthmannin (phosphatidylinositol 3-kinase inhibitor, 10  $\mu$ M), and mellitin-induced arachidonic acid release was significantly inhibited by bisindolmaleimide (protein kinase C inhibitor, 10  $\mu$ M) and worthmannin (10  $\mu$ M). These results indicate that phosphatidylinositol 3-kinase plays an important role in both arachidonic acid release and histamine release induced by mellitin in RBL 2H3 mast cells.

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[PB1-2] [ 04/18/2003 (Fri) 09:30 – 12:30 / Hall P ]