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We investigated Free radical generation of endocrine disruptor,
Bisphenol A and Alkyl esters of phthalic acid using lipid peroxidation, enzyme assay, MTT assay. Bisphenol A generated free radical, increased, lipid peroxidation, damaged antioxidant system and SK-MEL-28 Cell Line viability was dose-dependently increased. Also alkyl ester of phthalic acid generated free radical but slightly. The generation of free radical induced by endocrine disruptor was inhibited by antioxidant and free radical scavenger. The result of the study are demonstration on free radical induced by endocrine disruptor and this result may be useful for evaluating toxic effects of endocrin disruptor

[PA4-23] [04/20/2001 (Fri) 10:30 - 11:30 / Hall 4]

THE ROLES OF ATP AND CALCIUM IN MORPHOLOGY CHANGES AND CYTOTOXICITY INDUCED BY BENZOQUINONE IN PLATELETS

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To understand mechanism of benzoquinone-induced cytotoxicity, the roles of ATP and calcium in platelet toxicity and morphology changes was investigated. Using scanning electron microscopy, morphological changes to platelets following 1,4-benzoquinone exposure consisted of membrane blebbing at 5 min which was significantly different from shape changes (pseudopod formation) observed in response to physiological agonists. Benzoquinone-induced platelet membrane bleb formation was associated with rapid depletion of intracellular ATP and independent of presence of extracellular calcium. Benzoquinone-induced platelet lysis (LDH leakage) observed between 20-30 mins was dependent on extracellular calcium and associated with increased cytosolic calcium. Benzoquinone-induced cytotoxicity was inhibited by calmodulin antagonists, suggesting that calmodulin could play a major role in 1,4-benzoquinone toxicity via protease activation. These results suggested that the progression of events for quinone-induced cytotoxicity in platelets to be as follows: quinones deplete intracellular ATP; formation of blebs occurs; calcium homeostasis is disrupted, resulting activation of calmodulin-dependent proteases; irreversible cytotoxicity occurs.

[PA4-24] [04/20/2001 (Fri) 10:30 - 11:30 / Hall 4]

Suppression of cytochrome P450 1A1 in Mouse hepatoma Hepa-1c1c7 cells by o.p'-DDT

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Organo chlorine pesticides have received the most attention because of their persistence in the environment, ability to concentrate up the food chain, continued detection in the food supply and breast milk, and ability to be stored in the adipose tissue of animals and humans. In the present study we investigated the effect of op-DDT on TCDD-inducible Cytochrome (P450 1A1) gene expression in mouse hepatoma cell line Hepa-1c1c7 cells. Cultured mouse hepatoma Hepa-1c1c7 cells were treated with either o.p'-DDT or/and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or in combination to assess the role of o.p'-DDT in the process of P450 1A1 induction. TCDD-induced P450 1A1-specific 7-ethoxyresorufin O-deethylase (EROD) activity was markedly reduced in the concomitant treatment

of TCDD and o.p'-DDT in a dose dependent manner. TCDD-induced P450 1A1 mRNA level was also markedly suppressed in the concomitant treatment of TCDD and op'-DDT. Transient transfection assay using dioxin-response element (DRE)-linked luciferase revealed that o.p'-DDT reduced transformation of the aryl hydrocarbons (Ah) receptor to a form capable of specifically binding to the DRE sequence in the promoter of the P450 1A1. These results suggest the down regulation of the P450 1A1 gene expression by op'-DDT in Hepa-1c1c7 cells might be antagonism of the DRE binding potential of nuclear Ah receptor [This work was supported by KFDA Grant and RCPM from KOSEF].

[PA4-25] [04/20/2001 (Fri) 10:30 - 11:30 / Hall 4]

Species variation of neuropathy target esterase and its application to the neurotoxicity evaluation of pesticides

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Neuropathy target esterase (NTE) is an integral membrane protein in vertebrate neurons and plays an important role in neural development. Inhibition of more than 75% in NTE activity is associated with organophosphate-induced delayed polyneuropathy that is characterized by paralysis of the lower limbs and degeneration of long axons in the central and peripheral nervous systems. NTE activity was compared in three different species of hens, rats and mice and was applied to the neurotoxic evaluation of pesticides. NTE activity was determined by measurement of phenyl valerate esterase activity that is resistant to inhibition by paraoxon and sensitive to inhibition by mipafox. Tissue homogenates prepared from dissected brain regions in the three species were preincubated with inhibitors prior to phenyl valerate addition. Hydrolysis was stopped by protein denaturation and the production of the phenol content was spectrophotometrically determined. NTE activity was the highest in the cerebellum (2.10 ± 0.05 μmole/min/g tissue) in hens among three species, and was in significant decreasing order of whole brain (the entire brain regions except for the cerebellum and brainstem)> brainstem> spinal cord. In rats, NTE activity is lower than that in hens. Mice was the lowest in the activity among three species. This NTE activity was applied to pesticides that are not reported to NTE activity for neurotoxic evaluation.

[PA4-26] [04/20/2001 (Fri) 10:30 - 11:30 / Hall 4]

Environmental Estrogen effects on the TCDD stimulated CYP1A1 expression

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Environmental estrogens are studied recently in order to understand the health effects of them and the mechanism of action. CYP1A1 has been well known to be regulated by PAHs such as 3MC and TCDD. There are some possibility of crosstalk between TCDD and estrogen in terms of CYP1A1 expression. We have studied the two-way crosstalk between the arylhydrocarbon receptor (AhR) and estrogen receptor (ER) signaling pathways.
In our previous data, 17β-estradiol (E2) significantly inhibited TCDD-induced CYP1A1 gene expression and this inhibitory effect was partially recovered by concomitant treatment of tamoxifen. Like E2, 4-nonylphenol (NP), octylphenol (OP) and bisphenol A (BPA), known as 'Endocrine Disruptors', showed the estrogenic activities. In this study, we examined the effects of these chemicals on TCDD-induced CYP1A1 gene expression and CYP1A1 enzyme activity. And we investigated if their effects were mediated by ER signaling pathway. [This study has been supported by G7 from ME and HMP-98-B-3-0013]