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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.

[PA4-14] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

TCDD induced micronuclei in estrogen receptor positive human breast cancer cells

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Bisphenol A (BPA, Cas no. 80-05-7), di-2-ethylhexyl phthalte (DEHP, Cas no. 117-81-7), and 2,3,7,8-tetrachlorodibenzodioxin (TCDD, Cas no. 1746-01-6) were well known endocrine disrupting chemicals (EDCs). They showed all negative results in the Standard genetic toxicology test battery recommended by ICH guideline, i.e. bacterial reverse mutation assay, chromosome aberration assay, mouse lymphoma tk+/- assay and in vivo rodent micronucleus assay (MN). In our previous study, bisphenol A and di-2-ethylhexyl phthalte induced micronucleus formation in MCF-7 cells (estrogen receptor positive). In this study, to identify the relationship between MN formation and estrogen receptor (ER), TCDD was studied using micronucleus formation in human breast MCF-7 cells (ER positive). We also performed in vitro MN to identify the role of estrogen receptors with TCDD and tamoxifen, inhibitor of estrogen receptor, in MCF-7 cells during micronucleus formation. TCDD induced MN formation in MCF-7 cells (ER positive) was 6.60 fold higher than that of MCF-10A cells (ER negative). Though TCDD induced MN in MCF-10A cells, the frequencies were weak positive. Tamoxifen inhibited TCDD-induced MN formation up to 47.3% in MCF-7 cells.

[PA4-15] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

Potentiation of Agonist-Induced Platelet Aggregation by Trivalent Arsenic

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Chronic ingestions of arsenic by drinking water have been shown to induce cardiovascular disease

such as blackfoot disease, atherosclerosis and hypertension, but the exact mechanism has not been elucidated yet. In order to investigate one of the possible causes toward cardiovascular disease by arsenic, we examined the effects of arsenic on platelets which play an important role in development of cardiovascular disease. Addition of sodium arsenite (AsIII), trivalent inorganic arsenic, to rat platelets did not induce either aggregation or cytotoxicity to platelets directly, whereas AsIII treatment potentiated platelet aggregations induced by various agonists, such as thrombin, collagen, ADP and arachidonic acid in concentration- and time-dependent manners. Thrombin-induced platelet aggregation was also enhanced by relatively higher concentration of sodium arsenate (AsV) or monomethylarsonic acid (MMA) compared to AsIII. Treatment with AsIII resulted in a dose-dependent elevation of thrombin-induced serotonin levels from platelets, while the formation of thromboxane A2 from platelets did not altered significantly. Consistent with these findings, the in vivo studies revealed that ingestion of drinking water containing AsIII in mouse increased blood serotonin levels significantly, which is indicative of platelet aggregation in vivo. These results suggest that AsIII exposure makes platelets more susceptible to agonist-induced aggregation mediated through serotonin secretion from platelets and thus these effects by AsIII may contribute to the pathogenesis of cardiovascular disease.

[PA4-16] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

Studies on DNA damage by single cell gel electrophoresis and endocrine disrupting activity by transcriptional assay of dibutyl phthalate

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A wide range of phthalates have been produced for use as plasticizers and softeners in many synthetic products. Among phthalate esters, Di-n-butyl-phthalate (DBP) may act as xenoestrogens or antiandrogens. Also, DBP was reported to be genotoxic on human mucosa. To elucidate the relationship between endocrine disrupting activity and DNA damage of phthalate esters, DBP was studied by yeast-based steroid hormone receptor gene transcription assay and single cell gel electrophoresis. We have used a yeast-based assay to assess the interactions of DBP with the estrogen, androgen, and progesterone receptors. DBP ranging from 10^{-16} to 10^{-11} M was active in the estrogen receptor assay, but it did not show the effect on β-galactosidase activity in the progesterone and the androgen receptor assays. Also, to determine whether DBP induces DNA strand breakage, single cell gel electrophoresis (comet assay) was performed using mouse lymphoma L5178Y cell lines. The induction of strand breaks by DBP was not significantly different from control. In these assays, we found that DBP does not induce DNA single strand breakage in the single cell gel electrophoresis and DBP has estrogenic activity in the gene transcription assay of yeast-based steroid hormone receptor.

[PA4-17] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

Establishment of assay to screen estrogenic activity of chemicals

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To establish the rapid and easy-to-perform methods to screen estrogenic activity of many compounds, we determined 5'-ERE-regulated transactivation and cell proliferation in MCF-7 cells by luciferase assay and SRB assay, respectively. MCF-7 stable cells which are stably transfected with phERE-Luc were treated with many chemicals and then luciferase activity were determined. Estradiol (E2) and synthetic estrogen, diethylstylbesterol (DES) were induced luciferase activity in