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

dose dependent manner and their induced activities were decreased by tamoxifen (Tam) treatment. Phenolic compounds, such as octyl phenol (OP), nonyl phenol (NP), biphenol (BP), also induced the luciferase activity in dose dependent manner. Curcumin-derivatives, such as SB118, SB123, induced the luciferase activity and Tam treatment decreased SB118- and SB123-induced luciferase activities. Other curcumin-derivative, SB100, didn't induce the luciferase activity, but inhibited OP-, NP- and BP-induced luciferase activity. Over than 30 flavonoids were tested in this system, and isoflavone, such as biochanin A, daidzein, genistein, showed higher luciferase activity than others. Resveratrol driven from red wine induced the luciferase activity in dose dependent manner. To determine cell proliferative effect of chemicals, SRB assay was performed. E2 and DES increased the SRB readings 20-30 folds over that of control, and their activities were blocked by Tam treatment. Many flavonoids were tested in this system, and similar results to luciferase assay were achieved. These data shows that these methods are valuable tools for screening estrogenic activity of chemicals.

[PA4-18] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

Reduced generation of reactive oxygen species and proliferation in human neuroblastoma cells treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is one of the most toxic environmental pollutants. Wide range of toxic effects of TCDD have been known to be mediated through a ligand activated transcription factor termed arylhydrocarbon receptor (Ahr), which acts in concert with another structurally related protein, the arylhydrocarbon nuclear translocator. Despite the enormous reports regarding diverse actions of TCDD, the direct effect on the central nervous system has been largely unknown. In this study we have examined the toxic effects of TCDD on the human brain derived neuroblastoma cells. TCDD significantly suppressed proliferation of SK-N-SH cells. To elucidate the action mechanism, we studied possible involvement of reactive oxygen species and oxidative stress since endogenously generated reactive oxygen species are important growth modulatory signals. TCDD significantly reduced lipid peroxidation and generation of superoxide anion in the cells. The effect was not blocked by the treatment with α-naphthoflavone, a Ahr antagonist, or 8-methoxypsoralen, a binding inhibitor of activated Ahr to dioxin responsive element indicating that superoxide reducing action of TCDD is independent from its intracellular receptor. TCDD also significantly inhibited the activities of glutathione reductase, glutathione peroxidase. However, TCDD enhanced the activity of superoxide dismutase. In conjunction with the fact that a particularly risk group may be newborn infants, as it has been shown that TCDD is very efficiently transferred by lactation, the results suggest that TCDD may disturb brain development through inhibition of neuronal proliferation and generation of endogenous reactive oxygen species.
Supported by Korea Food & Drug Administration.

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

Gene expression profile and estrogenicity of dibutyl phthalate in MCF7 cells using cDNA microarray and E-screening test

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Various phthalate compounds are used as softeners and plasticizers in a wide range of plastic materials. Since these substances are not limited to the original products, but enter the