Our previous studies have demonstrated that nonenzymatic reaction of menadione with thiols in plasma generated reactive oxygen species, resulting in potentiation in the menadione-induced platelet toxicity. Since menadione, one of the representative quinone compounds, has been reported to cause hemolytic anemia in vivo, we hypothesized that erythrocytes could be one of the potential target tissues to menadione in the presence of plasma. To investigate the role of plasma in the erythrocyte toxicity by menadione and to identify reactive oxygen species derived from the nonenzymatic reaction of menadione with plasma thiols, plasma isolated from rats was treated with menadione sodium bisulfite (MSB), water soluble menadione. Treatment with MSB increased oxygen consumption rate as well as luminol- and lucigenin-amplified chemiluminescence in a dose-dependent manner. The chemiluminescences generated by luminol and lucigenin were inhibited by superoxide dismutase (SOD) addition, suggesting that superoxide anions were generated. When erythrocytes were suspended in plasma or buffer, MSB-induced chemiluminescence in plasma was larger than that in buffer, indicating that the presence of plasma increased free radical generation induced by MSB. Consistent with these findings, we observed MSB-induced hemolysis only in erythrocytes suspended in plasma while not in those suspended in buffer. In order to identify the reactive oxygen species associated with cytotoxicity, various radical scavengers were tested to inhibit MSB-induced hemolysis. Addition with catalase and/or mannitol resulted in significant inhibition of hemolysis, while superoxide dismutase had no effect. These results suggest that hydrogen peroxide and hydroxyl radical rather than superoxide appeared to be involved in erythrocyte cytotoxicity although the reaction of plasma thiols with MSB was accompanied by superoxide generation.

[PA4-2] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

A Study on Endocrine Disruptors: E-Screen Assay of Alkylphenolic Compounds

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It has been hypothesized that environmental estrogens may play roles in the increasing incidence of breast cancer, testicular cancer, and another problems of the reproductive systems. Alkylphenols which are widely used as plastic additives and surfactants have been shown to induce estrogenic responses. We tested 6 alkylphenolic compounds by E-screen assay. E-screen assay is suitable for large-scale screening of suspected endocrine disrupting chemicals. The method introduced by Soto *et al.* is based on proliferative activity of MCF-7 estrogen sensitive human breast cancer cell line. This quantitative cell proliferation assay of MCF-7 cells was performed in the absence and presence of 17β-estradiol (negative and positive controls), and at the range of various concentrations (10^{-14}~10^{-5} M) of alkylphenolic chemicals. Cell proliferation yields in the positive control increased up to six-eight fold over those of negative control cells after 144 hr incubation. Among the alkylphenols, 4-chlorophenol(10^{-5} M), cyclohexanol(10^{-13}~10^{-5} M) and 4,4'-isopropylidenediphenol (10^{-6}~10^{-5} M), α-naphthol(10^{-13}~10^{-12} and 10^{-7}~10^{-6} M), and p-nitrophenol(10^{-14} and 10^{-6}~10^{-5} M) appear to possess estrogen activity. And 4-buthylphenol showed week estrogenicity. The most potent estrogenic chemical was cyclohexanol which was able to stimulate these biological responses to the similar extent as 17β-estradiol itself.

[PA4-3] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

A Study on Endocrine Disruptors: E-Screen Assay of Newly Synthesized Plasticizer

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It is well known that environmental estrogens may play an important role in the increasing incidence of breast cancer, testicular cancer, and another problems of the reproductive systems. We tested newly synthesized plasticizer by E-screen assay. E-screen assay is suitable for large-scale screening of suspected endocrine disrupting chemicals. The method introduced by Soto *et al.* is based on proliferative activity of MCF-7 estrogen sensitive human breast cancer cell line. This quantitative cell proliferation assay of MCF-7 cells was performed in the absence and presence of 17β-estradiol (negative and positive controls), and at the range of various concentrations (10^{-14}~10^{-5} M) of newly synthesized plasticizer. Cell proliferation yields in the positive control increased up to six-eight fold over those of negative control cells after 144 hr incubation. Among the newly synthesized plasticizer, KH005(10^{-11}~10^{-9} M), KH008(10^{-13}~10^{-5} M), KH010(10^{-14}~10^{-6} M) and KH011(10^{-9}~10^{-5} M) appear to possess estrogenic activity about 2~6 fold. And KH001(10^{-7}~10^{-5} M) showed week estrogenicity (1~2 fold). The most potent estrogenic chemical was KH 008(10^{-13}~10^{-5} M), which was able to stimulate these biological responses to the similar extent as 17β-estradiol itself, albeit at a 10^{-5} M fold greater concentration than 17β-estradiol. KH001(10^{-14}~10^{-5} M) exihibited very week estrogenicity. Futher study such as molding capacity for the development of new plasticizer is undergoing.

[PA4-4] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

Cytokine-mediated Induction of Metallothionein by α-Hederin in Macrophages

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α-Hederin, a triterpenoid saponin which exists in some oriental herbs, was known to decreased the hepatotoxicity of cadmium by inducing expression of the metallothionein. However, the mechanism(s) by which α-Hederin induces metallothionein is not well investigated. In the present study, we investigate the role of inflammatory cytokines in α-Hederin-induced up-regulation of the metallothionein in murine peritoneal macrophages. The induced expression of metallothionein in α-Hederin-treated cells was accompanied by increase of productions and transcripts for IL-1β, IL-6, and TNF-α in a dose-dependent manner by immunoassay and RT-PCR analysis, respectively. Since the promoter in IL-1β, and TNF-αgene contains binding motifs for NF-kB, the effect of α-Hederin on the activation of this transcription factor where determined. Using DNA fragments containing the NF-kB binding sequence, α-Hederin was shown to activate the protein/DNA binding of NF-kB to its cognate site as measured by electrophoretic mobility shift assay. Collectively, the results of these experiments indicate that expression of IL-1β, IL-6, and TNF-α were induced by α-Hederin and these results show that the inflammatory cytokines that are induced by α-Hederin may play an important role in the α-Hederin-induced up-regulation of metallothionein. [Supported by KOSEF Grant 1999-2-214-001-5]