Toxic bile salts are known to exert hepatocyte toxicity by inducing apoptosis. Although it has been reported that Fas- death receptor pathway is the predominant pathway of apoptosis in cholestasis, the precise mechanism of bile salt-mediated apoptosis is still not fully understood. We investigated cellular localization and expression of proteins involved in the bile salt-mediated apoptosis in bile duct-ligated (BDL) rats by immunohistochemistry and Western blot analysis. Activated stellate cells, responsible for liver fibrosis, were ncreased in portal and periductular areas in BDL rats over 8 weeks. In sham controls, Fas was weekly expressed in cytoplasm of hepatocytes but not in bile duct epithelial cells (BEC). The expression was inhanced by BDL for first 3 days, and remained constituitively expressed over 8 weeks. Bax was expressed in a punctate manner indicative of mitochondrial localization in BEC and hepatocytes of sham controls. Bax was increased by BDL, and then its expression was decreased with time. Antiapoptotic protein Bcl-2 was detected only in BEC of sham controls. At day 3 after BDL, de novo Bcl-2 expression was observed in hepatocytes, and the strong immunoreactivity was observed in hepatocytes located along the bile ductules. After BDL for two weeks, expression of Bcl-2 showed a marked increase in BEC and also showed strong expression in periportal hepatocytes. Expression pattern of p53, a transcription factor, was very similar to that of Bax expression. We demonstrated that Fas was strongly expressed in the cytoplasm of hepatocytes in BDL rats, indicating the involvement of soluble Fas molecules. Expression pattern of Bax showed a good inverse correlation with that of Bcl-2 expression. Also expression of Bax may be regulated by p53.

[PA3-13] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

Comparison of corresponding human intake giving biochemical toxicity induced by TCDD in risk Assessment

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The most sensitive biochemical effect dioxin and related chemicals are CYP1A1/2 induction, EGF-receptor down regulation and oxidative stress. Currently, the body burden giving rise to specific toxicological endpoint has been used for calculation of human external intake in dose-response assessment in field of risk assessment improved by PB-PK model. The animal body burden giving rise to statistically significant effect related with CYP1A1/2 induction, and EGF receptor down-regulation induced by TCDD using animal data have reported as range of 3~10ng/kg by WHO (1998). U.S.EPA(2000) suggested corresponding animal body burden (0.17~12.3ng/kg) giving biochemical toxicity like CYP1A1/2 induction and EGF receptor down regulation from dose-response model. This study has compared difference of above two value using conversion equation which can human intake level from animal body burden : Intake(ng/kg/day) = body burden(ng/kg)×ln2 / half-life×absorption rate (ln2=0.693, half-life 7.5years, absorption rate 50%). The range of corresponding human intake giving biochemical toxicity based on WHO and U.S.EPA data were 1.52~5.06pg/kg/day and 0.086~6.23pg/kg/day, respectively.
If above two values regard and apply uncertainty factor 10 (human variability), tolerable daily intake 0.009~0.62pg/kg/day can be used as possible human intake without giving rise to biochemical toxicity induced by TCDD.

[PA4-1] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

The Role of Rat Plasma in Manifesting Toxicity of Erythrocytes by Water -Soluble Menadione: Further Evidence of Free Radical Generation

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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]]