The Study on Screening for Endocrine Disrupting Property of Alkylphenolic Compounds

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Alkylphenols are widely used as plastic additives and surfactants. The estrogenic properties of alkylphenols may lead to spurious results, and may also be potentially harmful to exposed humans and the environment at large. The purposes of our study were to screen and elucidate the endocrine disrupting activity and mechanism of 12-alkylphenols. Among these compounds, p-nitrophenol (Maximal Response, 105.3%) and was the most active in the yeast estrogen transcriptional assay (YES), followed by the 4-tert-octylphenol (MR, 91.3%) and bisphenol A(MR, 88.4%). In yeast progesterone (YPS) and androgen (YAS) transcriptional assay, p-nonylphenol and isocyanic acid- chlorophenol showed a weak response (MR, 4.68% and 3.36%) respectively. In vitro E-screen cell proliferation assay, bisphenol A, 4-tert-octylphenol, and p-nonylphenol showed high estrogenic activity. Their relative proliferation efficiency(RPE) were 110.3, 104.5 and 107.3%, respectively. In vitro estrogen receptor(ER) competitive binding assays, their relative binding affinity is 0.0143, 0.1968, and 0.0739%, respectively. In vivo uterotrophic assay, these three chemicals increase uterus weight in immature rats about 1.4, 2.7 and 1.7-fold compared to control. As a result, 4-tet-octylphenol, p-nonylphenol, and bisphenol A possess strong estrogen activities. And these assessment methods can be useful screening environmental chemicals for endocrine disrupting activity.

[PA4-8] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

The estrogenic effects of pyrethroid insecticides using glucose-6-phosphate dehydrogenase in MCF-7 cells and uterine glutathione peroxidase in rats

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The synthetic pyrethroid insecticides are now the most widely used agents for indoor control, providing potential for human exposure. We have shown that the glucose-6-phosphate dehydrogenase (G6PD) in estrogen sensitive human breast cancer cells (MCF-7 cells) and glutathione peroxidase (GPx) in female immature Sprague-Dawley rats could be used as useful screening methods for endocrine disruptors. In this study we have endeavored to screen permethrin, cypermethrin and phthalthrin for estrogenic effects using assays of uterine GPx and G6PD in MCF-7. For GPx assay, 19-day female rats were treated with pyrethroids (permethrin, PM, cypermethrin, CM, phthalthrin, PT, 100, 500 1,000 mg/kg, s.c., 3 days), or 17β-estradiol (0.3 mg/kg, s.c., 3 days) as a positive control. GPx activities in uterine were significantly enhanced at the high doses (1,000 mg/kg) of PM, CM, and PT without increases in uterine weights. Also at dosages of 100 and 500 mg/kg, PM, CM and PT tended to increase GPx activities, while these changes were not statistically significant. These results suggest that permethrin, cypermethrin, and phthalthrin have estrogenic effects and might act as endocrine disruptors. The G6PD assay in MCF-7 is under study.

[PA4-9] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Role of a 42 kDa Ca^{2+}-dependent Cytosolic Phospholipase A_2 in RBC Function

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It become evident that Ca^{2+}-dependent release of arachidonic acid (AA) and subsequent formation of bioactive lipid mediators such as prostaglandins and leukotrienes in red blood cells (RBCs) can mofify physiological functions of neighboring RBCs and platelets, and thus influence hemostssis and thrombosis. Here we identified a novel type of cytosolic PLA_2 in bovine and human RBCs and purified it to apparent homogeneity with a 14,000-fold purification. The purified enzyme, termed $rPLA_2$, had a molecular mass of 42 kDa and revealed biochemical properties similar to group IV $cPLA_2$, but showed different profiles from $cPLA_2$ in several column chromatographies . Moreover, $rPLA_2$ did not react with any of anti-$cPLA_2$ and $sPLA_2$ antibodies and examined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis. Divalent metal ions tested exhibited similar effects between $rPLA_2$ and $cPLA_2$, whereas mercurials inhibited $cPLA_2$ but had no effect on $rPLA_2$. Antibody against the 42 kDa protein not only precipitated the $rPLA_2$ activity, but also reacted with the 42 kDa protein from bovine and human RBC in immunoblot analysis. The 42 kDa protein band was selectively detected in murine fetal liver cells as a type of progenitor cells of RBCs. Finally, we found that EA4, a derivative of quinone newly developed as an inhibitor for $rPLA_2$ in vitro, inhibited a Ca^{2+} ionophore- and mechanical stress-induced AA release from bovine and human RBCs. Furthermore, Ca^{2+} ionophore- and exogenous AA-induced AA release inhibited by PD 98059, MAP kinase inhibitor. Our results altogether demonstrate that the 42 kDa $rPLA_2$ is identified as a novel form of Ca^{2+}-dependent PLA_2 and responsible for the Ca^{2+}-dependent AA release from human and bovine RBCs and modulated by MAP kinase at least in part.

[PA4-10] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Protective effects of paeonol on cultured rat hepatocytes exposed to Br-A23187

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Br-A23187 (Ca2+ ionophore) is frequently used as a model of Ca2+-dependent cell killing as Br-A23187 induces both necrotic and apoptotic cell death. The aim of this study was to evaluate the protective effects of paeonol isolated from Paeoina Moutan on cultured rat hepatocytes exposed to Br-A2187. Cell killing was assessed propidium iodide fluorometry. Br-A23187 caused dose-dependent cell killing. Br-A23187 (10μM)-induced cell killing was decreased in the presence of paeonol (20, 50 and 100 μM). On the other hand, Br-A23187-induced [Ca+2]i level was increased dose-dependently. However, paeonol decreased Br-A23187-induced [Ca+2]i level in dose-dependent manner. Additional measurements of enzyme activities were made to know whether paeonol inhibits those enzyme activities such as LDH, S-GOT and S-GPT on cultured hepatocytes.
Therefore, the present results indicate that paeonol has protective effects against Br-A23187-induced hepatocytotoxicity in rats, indicating paeonol decreases [Ca+2]i level and inhibits enzyme activities related to hepatotoxicity.

[PA4-11] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

The Screening of Toxicity from the Processed Aconiti Tuber in vitro and in vivo

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