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In this study, we investigated the effect of bisphenol A on the regulation of iNOS and proinflammatory cytokines, such as, IL-6 and TNF-αin mouse peritoneal macrophages. Bisphenol A alone did not affect the expression of iNOS and proinflammatory cytokines; in contrast, treatment of bisphenol A suppressed the LPS-induced gene expression of IL-6, TNF-α and iNOS, in a dose-dependent manner as determined by RT-PCR analysis. Bisphenol A was shown increased TNF-α gene expression in a dose-dependent manner. NO production was assessed by measurement of nitrites in the medium. The level of NO was found to correlate well with a decrease in transcripts of iNOS. Since the promoter in TNF-α and iNOS gene contains binding motifs for NF-kB, the effect Bisphenol A on the inactivation of this transcripts factor was determined by transient transfection assay and electrophoretic mobility shift assay (EMSA). Employing a transfection and reporter gene expression system with p(NF-kB)3-Luciferase, the treatment of bisphenol A produced a dose-dependent inhibition of luciferase activity in RAW 264.7 murine macrophages cell line. Using DNA fragments containing the NF-kB binding sequence, nonylphenol was shown to inhibition the protein/DNA binding of NF-kB to its cognate site as measured by EMSA. These results suggest that suppression of iNOS and IL-6 gene expression by bispheonl A might be mediated by the inhibition of NF-kB activation [This work was supported by KFDA Grant and RCPM from KOSEF].

[PA4-32] [04/20/2001 (Fri) 10:30 - 11:30 / Hall 4]

Down-regulation of iNOS, TNF-a and IL-6 gene expression by genistein in murine macrophages.

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The phytoestrogen and principal isoflavone in soy, genistein, has adverse effects on reproductive physiology in rodents. Therefore, in this study, we investigated the effect of genistein on the of iNOS and proinflammatory cytokines, such as, IL-6 and TNF-αin murine macrophages. Genistein alone did not affect the expression of iNOS and proinflammatory cytokines; in contrast, treatment of genistein suppressed the LPS-induced gene expression of IL-6, TNF-αand iNOS, in a dose-dependent manner as determined by RT-PCR analysis. NO production was assessed by measurement of nitrites in the culture medium. The level of NO was found to correlate well with a decrease in transcripts of iNOS. Since the promoter in TNF-αand iNOS gene contains binding motifs for NF-kB, the effect genistein on the inactivation of this transcripts factor was determined by transient transfection assay. Employing a transfection and reporter gene expression system with p(NF-kB)3-Luciferase, the treatment of genistein produced a dose-dependent inhibition of luciferase activity in RAW 264.7 murine macrophages cell line. These results suggest that suppression of iNOS, TNF-α, and IL-6 gene expression by genistein might be mediated by the inhibition of NF-kB activation [This work was supported by KFDA Grant and RCPM from KOSEF].

[PA4-33] [04/20/2001 (Fri) 10:30 - 11:30 / Hall 4]

Down-regulation of murine Cyp1a-1 in mouse hepatoma Hepa-1c1c7 cells by genistein

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The phytoestrogen and principal isoflavone in soy, genistein, has adverse effects on reproductive physiology in rodents. Cultured mouse hepatoma Hepa-1c1c7 cells were treated with either genistein or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or in combination to assess the role of genistein in the process of cytochrome P450 1A1 (Cyp1a-1) induction. Treatment of Hepa-1c1c7 cultures with TCDD induced Cyp1a-1, as indicated by analysis of 7-ethoxyresorufin O-deethylase (EROD) activities. Genistein alone did not affected the activity of Cyp1a-1-specific EROD activitys; in contrast, TCDD-induced EROD activities were markedly reduced in the concomitant treatment of TCDD and genistein in a dose dependent manner. Treatment with tamoxifen, an antiestrogen that acts through the estrogen receptor did not affect the suppressive effects of genistein on TCDD-induced EROD activity. TCDD-induced Cyp1a-1 mRNA levels were markedly suppressed in the concomitant treatment of TCDD and genistein consistent with EROD activity. Transient transfection assay using dioxin-response element (DRE)-linked luciferase revealed that genistein reduced transformation of the aryl hydrocarbons (Ah) receptor. These results suggest the down regulation of the Cyp1a-1 gene expression by genistein in Hepa-1c1c7 cells might be antagonism of the DRE binding potential of nuclear Ah receptor but not through estradiol receptor [This work was supported by KFDA Grant and RCPM from KOSEF].

[PA4-34] [04/20/2001 (Fri) 10:30 - 11:30 / Hall 4]

INTERNATIONAL COOPERATION STUDY FOR EVALUATION OF OECD SCREENING AND TESTING METHOD FOR ENDOCRINE DISRUPTORS

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The uterotrophic and Hershberger assays are considered as potential short term screening assays to detect the endocrine disruptors by OECD and EDSTAC. The OECD is in the process of developing these assays and validating its protocols by participating laboratories carry out the work according to each of the designed protocols. Preliminary findings suggest that these assays are robust and ready for protocol standardization and inter-laboratory validation. Therefore, the objective of this study was to provide data for the standardization of the OECD rodent uterotrophic assay (second stage) and Hershberger assay (first-stage). In the 3-day uterotrophic assay using immature female rats, neither BA and BE treatment affected mean uterus (wet and blotted) weights. However, a significant increases in mean uterine wet weights were observed in BF (775% of VC), BB (392% of VC), BC (367% of VC), BD (249% of VC), BH (232% of VC) and BG (180% of VC) groups. The changes of uterine blotted weights showed a similar pattern with uterine wet weight. Also, dose-related estrogenic effects of bisphenol-A and nonylphenol were observed. In bisphenol-A treatment groups, dose-related increases in both mean uterine wet and blotted weights were observed. Moreover, these increases were statistically significant at doses of 300 mg/kg/day and above. Nonylphenol also statistically significant increases of uterine wet and blotted weights were observed at doses of 80 mg/kg/day and above. Hershberger assay was performed utilizing immature Sprague-Dawley male rats castrated at 6 weeks of age. Testosterone propionate (0.1, 0.2, 0.4, 0.8 and 1.6 mg/kg/day) was subcutaneously (s.c.) injected for 10 days. Additionally, a pure androgen antagonist, flutamide (1, 5, and 10 mg/kg/day) was administered by oral gavage after testosterone treatment. In testosterone propionate, glans penis (GP), seminal vesicles (SV), ventral prostate (VP), levator ani muscle and bulbocavernous muscle (LABC), and cowper's glands (CpG) weights were significantly and dose-dependently increased. Flutamide inhibited the testosterone-induced re-growth of accessory sex glands (SV, VP and Cp) and organs (GP and LABC) with dose-dependent manner. These results suggests that uterotrophic and Hershberger assays may be useful methods for detecting the endocrine disruptors with estrogenic/antiestrogenic or androgenic/antiandrogenic activity.