saturation of the first-pass metabolism together with concomitant saturation of hepatic clearance in the metabolism of fenfluramine.

[PA4-2] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Simple Screening Method of Endocrine Disruptors using spot-test procedure of yeast-based steroid hormone receptor gene transcription assay

Jung HK, Park MS, Lee JJ, Park HS, Kim JW, Eom MO, Han ES, Jeong SH, and Oh HY

Toxicology Department, National Institute of Toxicological research, KFDA

Simple Screening Method of Endocrine Disruptors using Spot-test procedure of Yeast-based Steroid Hormone Receptor gene transcription assay

HK Jung, MS Park, JJ Lee, HS Park, JW Kim, MO Eom, ES Han, SH Jeong,and HY Oh
Toxicology Department, National Institute of Toxicological Research, KFDA

A yeast-based steroid hormone receptor gene trascription assay was previously developed for the evaluation of chemicals with endocrine modulating activity by McDonnell's group at Duke University Medical Center, USA. The yeast transformants used in this assay contain the human estrogen, androgen or progesteron receptor along with the appropriate steroid responsive elements upstream of the β-galactosidase reporter gene. The original procedure of the assay comprised the following step: i) Dilution of early mid-log phase culture to an OD600 of 0.03 in selective medium plus CuSO4 to induce receptor production ii) Addition of either steroid or test chemical, followed by overnight incubation with shaking iii) Dilution to OD600 of 0.25 and aliquotes of 100㎕ added to 96-well microtiter plate iv) Addition of equal volume of assay buffer containing 2-nitrophenol-β-D-galactosidase(ONPG) as a substrate for β-galactosidase v) Measurement the change in concentration of orthonitrophenol using a microtiter plate reader. We here report a simple spot-test procedure using X-gal as a substrate for β-galactosidase instead of ONPG. Production and induction of β-galactosidase can be evidenced on plates containg X-gal which released a colored dye when hydrolyzed by β-galactosidase. Effect of the variation of the medium components and oxalyticase application on the response in this spot assay will also be discussed.

[PA4-3] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Stability Study on DK-35C, a Carbapenem Antibiotics by HPLC : Effects on pH and Time Changes

Kwon OS[o], Rhee HK, Kim MS, Kim MK[2], Nam KH[2] and Ryu JC

Toxicology Laboratory, Korea Institute of Science & Technology P.O. Box 131, Cheongryang, Seoul 130-650, Korea, [2]Dong-Kuk Pharmaceutical Company, Chungbuk, Korea

Impurity profile study of DK-35C (a carbapenem antibiotics) was conducted by two different methods of HPLC/UV. In Experiment I, Bondapak C18 column and 0.01 M ammonium phosphated buffer with 0.05% triethylamine (pH 6.5)/methanol (85/15, v/v) as mobile phase were used. Parent and its related impurity peaks were monitored for 6 days. In Experiment II, Lichrosorb RP18 column and sodium phosphate buffer (pH 6.5)/methanol (7/3, v/v) as mobile phase were used. Peak areas of DK-35C and its impurity was measured at several different pH values. The result from Experiment I is that DK-35C parent (11.9 min) and four impurity peaks (3.3, 5.8, 10.1, and 19.1 min) were observed and methanol solution of DK-35C showed a rapid degradation after 24 hrs. The maxium wavelength of DK-35C absorption was observed at 300 nm. The results from Experiment II showed

that DK-35C is relatively stable at pH ranges of 5-7.4, compared to other pH solutions (pH 2, 9, and 11) used, and peak area of DK-35C is decreased as time elapses, suggesting that parent and impurity profiles of DK-35C are pH- and time- dependent.

[PA4-4] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

The retinoic acid and cyclophosphamide treatment altered the Hoxa-7 gene expression in limb buds and tail during mouse development

Kim HS°, Han SY, Lee DH, Shin JH, Moon HJ, Kim SH, Kim TS and Park KL

Reproductive and Developmental Toxicology Division, National Institute of Toxicological Research, Korea Food and Drug Administration

Homeobox-containing (HOX) genes are known to be involved in pattern formation during development by expressing in a stage-, position-, and tissue-specific manner. Among Hox genes, Hoxa-7 is one of the well known genes, which expressed in the ectoderm-derived neural tube and neural crest cells, and mesoderm-derived prevertebrae along the anterior-posterior axis. However, it is not clear whether Hoxa-7 gene is directly involved in mouse embryonic limb and tail development. In this study we examined Hoxa-7 gene expression by reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting in the developing limb- and tail buds of ICR mice embryos after maternal administration of retinoic acid (all-trans RA) and cyclophosphamide (CP) on 10 days of gestation. Morphologically, the RA treatment at the concentration of 100 mg/kg caused shortening of forelimbs and deletion of tail. Also, CP (20 mg/kg) markedly induced the anomalities of limb, i.e., syndactylia and polydactylia. RT-PCR and western blot analysis indicated that Hoxa-7 gene was highly expressed in hind limb and tail compared to forelimb during normal embryogenesis. However, Hoxa-7 gene expression was increased in both tail and hindlimb of gestation day 13 embryos by RA treatment. In the case of CP, Hoxa-7 gene expression was highly increased in both forelimb and hindlimb, but not tail. Therefore, these results altogether suggested that Hoxa-7 gene may play an important role in the morphological changes of limd and tail during embryogenesis.

[PA4-5] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Cytogenetic, DNA strand breakage and forward gene mutation studies of higenamine, a constituent of Aconite sp., in mammalian cells

Kim HT°[1], Yun HS[2], Chang KC[3], Lee DH[4], Ryu JC[1]

[1]Toxicology Laboratory, Korea Institute of Science and Technology, Seoul, 136-650, Korea, [2]NPRI, Seoul National University, [3]Gyeongsang National Univ. College of Medicine, [4]Dept. Chemistry, Sogang Univ.

To investigate the toxicity of higenamine, a constituent of Aconite *sp.*, we performed the thymidine kinase forward gene mutation assay, single cell gel electrophoresis with L5178Y mouse lymphoma cells, and cytokinesis blocking micronucleus assay with V79 cells. In forward gene mutation assay with L5178Y mouse lymphoma cells, higenamine revealed statistically significant increases in mutation frequency. However, in the single cell gel electrophoresis and cytokinesis blocking assay, higenamine did not have statistically significant difference in DNA strand-breakages induction and binucleated micronucleus formation. From these results, higenamine may be involved in some point mutation and small scale of DNA damages at the high concentration, but did not induced the large scale DNA strand-breakage and micronuleus formation.

[PA4-6] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]