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

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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

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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]]