RESEARCH REPORT - No. 1104 FY 2011–2013

Title of research project	Development of combined genotoxicity and repeated dose toxicity study in rats
Research project no.	(1104)
Research period	FY 2011–2013
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【Abstract】

A combined genotoxicity and repeated-dose toxicity study has been developed. To standardize the study protocol, study duration and strain difference were examined, and applicability to 13-week repeated-dose toxicity was investigated. In addition, to elucidate the effect of transgene λEG10 on genome, the inserted site was determined. Furthermore, the effects of aging on accumulation of mutation and clonal growth of mutants were also examined.

F344 and SD *gpt* delta rats were given a liver genotoxic carcinogen diethylnitrosamine (DEN) for up to 2 to 8 weeks in drinking water. Treatment with the highest dose 10 ppm significantly increased *gpt* mutant as well as Spi^- deletion frequencies in the liver at each time point compared to the non-treatment control. In contrast, a liver non-genotoxic carcinogen di(2-ethylhexyl)phthalate feeding did not affect *gpt* mutant nor Spi^- deletion frequency.

F344 *gpt* delta rats were given DEN for 13 weeks in drinking water. Repeated-dose toxicity findings including general condition, serum biochemistry, organ weight and histopathology were comparable to those of wild F344 rats. The precancerous hepatic foci were significantly induced with the treatment although there was no difference between *gpt* delta and wild rats in terms of induction capability.

F344 *gpt* delta rats were intraperitoneally given DEN for 5 weeks and then orally phenobarbital for 8 weeks. Mutation profiles of *K-ras* were coincident with those of *gpt* confirmed by mutation spectrum analysis, suggesting that mutation on *gpt* gene could be also involved in oncogenes.

It was found that only one gene is included in the deletion portion caused by insertion of λEG10. From comparable data between *gpt* delta and wild rats in terms of repeated-dose toxicity, such deletion would not affect hazard evaluation. Spontaneous mutation was accumulated with age, and spectrum analysis for the increased mutations suggested possible oxidative DNA modification. In comparison with 3 strains, there was no difference in spontaneous mutation frequency in the liver of F344, SD and Wistar Hannover rats.

It is thus concluded that *gpt* delta rats are applicable to repeated-dose toxicity study without influence of the transgene. In some chemicals, genotoxicity could be evaluated in periods shorter than 4 weeks. A combined genotoxicity and repeated-dose toxicity using *gpt* delta rodents would be definitely innovative for acceleration and sophistication of current toxicity studies.