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Author(s)	嶋田, 圭祐
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学位論文内容の要旨
Abstract of the dissertation

博士の専攻分野の名称：博士（獣医学）　　氏名：嶋　田　圭　祐
Name

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The title of the doctoral dissertation

A study of micronucleated hepatocytes detection in the liver micronucleus assay using young adult rats（成熟ラットを用いた肝臓小核試験における小核を有する肝細胞の検出に関する研究）

A repeated-dose liver micronucleus (RDLMN) assay of young adult rat was recently developed to evaluate the genotoxic hepatocarcinogens. For evaluating the effectiveness of this assay, I performed the RDLMN assay in young adult rats that received intraperitoneal injections of 0.25, 0.5 and 1.0 mg/kg/day of mitomycin C (MMC) for both 14- and 28- day periods. The micronucleus induction in the bone marrow was concurrently measured, and a histopathological examination of the liver was conducted. The results revealed that the frequency of micronucleated hepatocytes (MNHEPs) was significantly increased in all of the treatment groups. However, the highest occurrence of MNHEPs was observed in the low-dose treatment group in both the 14- and the 28-day study periods despite the frequency of micronucleated immature erythrocytes in the bone marrow significantly increased in a dose-dependent manner in all of the treatment groups in both study periods. In addition, histopathological changes indicating hepatotoxicity were not observed even in the group that received the highest dose of MMC. There was no change in the frequency of mitosis phase hepatocytes in any of the treatment groups compared with facility's background data. However, the frequency of proliferating hepatocytes, as assessed by Ki-67 positivity, was decreased at the highest dose, as was the frequency of MNHEPs. Therefore, the RDLMN assay can detect the genotoxicity of MMC, and the decreased induction of MNHEPs in the high-dose groups may be explained by suppression of hepatocyte cell division.

In the RDLMN assay, accumulation of MNHEPs induced by repeated dosing of

genotoxic chemicals is considered a key factor in the detection of micronuclei induction. Then, I hypothesized that the period following chemical exposure enables the detection of MNHEP induction in young adult rats, namely that MNHEPs can be generated from chromosomally damaged cells and accumulate following initiation of chemical exposure until sampling. I therefore measured MNHEP induction at 2 and 4 weeks after a single oral administration of 12.5, 50 and 100 mg/kg of diethylnitrosamine (DEN) and an intraperitoneal administration of 0.5, 1.0 and 2.0 mg/kg of MMC to young adult rats. Results showed a statistically significant and dose-dependent increase in the number of MNHEPs in both DEN- and MMC-treated rats, indicating that prolonged rest period following a single dose of a genotoxic chemical enables the detection of MNHEP induction in the liver of young adult rats. From these results, a single oral administration of 50 mg/kg of DEN with a 2- and 4- week rest period can be used as a positive control in RDLMN assays. This procedure is superior in terms of labor saving and animal welfare to repeated dosing of DEN. In addition, single-dose liver micronucleus assay with 14-day rest period using young adult rats may also be available for a regulatory genotoxicity test.