P-55

Response to the Issues Raised at the 7th IWGT Meeting: Effect of Aging on the Repeated Dose Liver Micronucleus Assay

○ Miyuki Shigano[1], Kiyoko Nakadate[1], Hironao Takasawa[1], Shuichi Hamada[1]

[1]LSI Medience Corporation, Kamisu, Ibaraki, Japan

Abstract

The liver micronucleus (MN) assay is an effective and important in vivo test for detecting genotoxic compounds, particularly for those that require metabolic activation to show genotoxicity. We have developed a repeated dose liver MN (RDLMN) assay which greatly facilitates incorporation of a liver MN assay into a general toxicity study. Usefulness of the RDLMN assay was appraised highly in the 7th International Workshops on Genotoxicity Testing (7th IWGT, 2017 in Tokyo). The working group members agreed that the RDLMN assay is sufficiently validated for an OECD guideline in terms of numbers and types of chemicals studied, and the easy integration into general toxicity studies is preferred from the 3R's point of view; however, it is necessary to evaluate the impact of dosing animals of different ages (6- versus 8-weeks old). This is because the age of animals at the start of administration in a 4-week repeated dose general toxicity study often differs between Japan (6 weeks) and Western countries (8 weeks). Our data were for animals at 6 weeks of age at the start of administration only, but not for animals at 8 weeks of age. In this study, we used clofibrate, a weak inducer of the liver MN, to compare the age difference between 6 and 8 weeks at the start of administration to examine effects of aging on the results of RDLMN assays, and the results are to be reported at the ACEM/JEMS meeting.

Background 1

【 Effects of repeated dosing on micronucleus assay using hepatocytes 】

2,4-DAT is a hepatocarcinogen but difficult to detect by a bone marrow micronucleus assay. Even in juvenile rats which have high mitotic activity, MNHEP induction did not increase more than 2-fold that of the negative controls in a double treatment. On the other hand, a 28-day repeated treatment showed remarkable acceleration in micronucleus induction by the accumulation of MNHEP even in mature rats which have low mitotic activity.

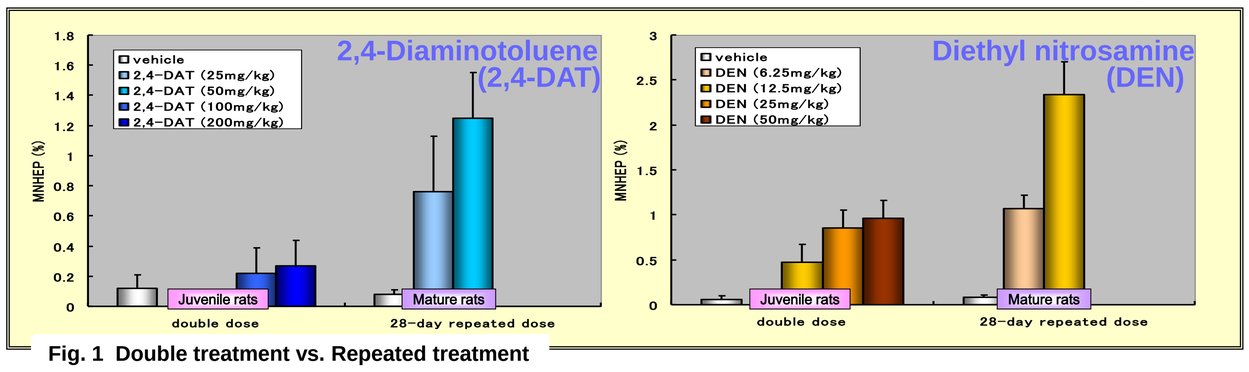

Fig. 1 Double treatment vs. Repeated treatment

【 Accumulation and time course of liver micronucleus induction 】

In both the 2,4-DAT and DEN administration, MNHEP(%) was the highest in the 28-day repeated dose, followed by the 14-day and 5-day repeated dose in this sequence, which suggesting accumulation of the MNed hepatocytes. These results suggest that MNHEP is not selectively eliminated, but accumulated after repeated dose for a general cellular life span, about 200 days. The liver MN assay is extremely effective when integrated into repeated dose general toxicity studies, taking advantages of hepatocytes long-term viability (180 to 200 days) that brings about accumulation of MNed hepatocytes and good compatibility with repeated treatment even in a low top dose (not depend on Cmax).

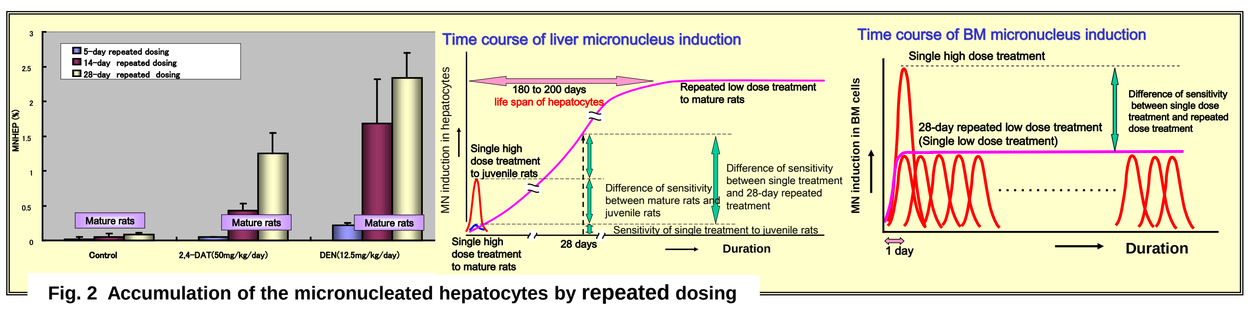

Fig. 2 Accumulation of the micronucleated hepatocytes by repeated dosing

Background 2

【The performance of liver MN assay 】

Follow-up studies to the test performance have been performed after the 6th IWGT, and the results were reported at the 7th IWGT. Four aneugens (carbendazim, colchicine, vinblastine and docetaxel), 2 genotoxic non-carcinogens (amaranth and 2,6-diaminotoluene), and 3 non-genotoxic non-carcinogens (alpha-naphthyl isothiocyanate (as a hepatotoxicant), sodium chloride and sucrose) were newly examined with the repeated dose method of liver MN assay (Fig. 3). Carbendazim, vinblastine, and docetaxel were found positive, and colchicine was equivocal in the 4 days treatment but negative in the 28 days treatment. It was considered that colchicine failed to increase liver MN because it showed strong mitotic inhibition under the test conditions (i.e., dosing period and dose levels) of the 28 days treatment. Overall, these results suggest that aneugenic chemicals can be detected by the liver MN assay with appropriate dosing period and dose levels. The two genotoxic non-carcinogens, and the 3 non-genotoxic non-carcinogens, including a hapatotoxicant, were found negative in the MN assays, indicating that the liver MN assay would have high specificity for carcinogenicity.

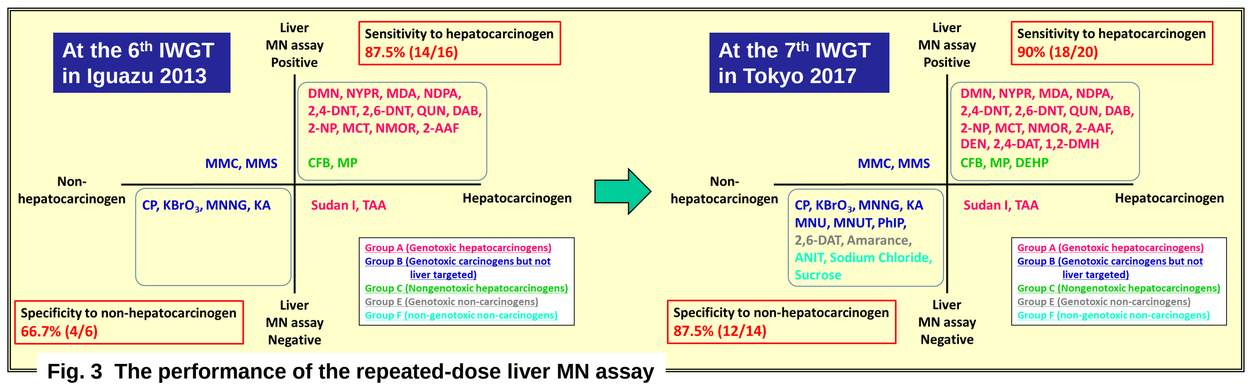

Fig. 3 The performance of the repeated-dose liver MN assay

【The agreements and the issues pointed out at the 7th IWGT meeting】

1. **The working group members agreed that the liver MN assay is sufficiently validated in terms of numbers and types of chemicals studied to develop this test into an OECD Test Guideline.**
2. **From a 3R's point of view, combination with other genotoxicity assays and/or integration into general toxicity studies would be preferred.**
3. **Regarding integration potential, it should be noted that the OECD 407 Guidance (rat one month study) indicates that dosing should begin as soon as feasible after weaning, in any event before 9 weeks of age.**
4. **This recommendation therefore supports the use of 6-week old rats, the age that was used to develop the majority of one month repeated dose liver MN assays have been accomplished to date. However, given the tendency of industry to conduct their general toxicology studies with rats older than 6 weeks at start of treatment, the impact of age at the time of dosing warrants further study.**

Effects of aging

【 Animals 】

We used male Crl:CD(SD) rats that were purchased from Charles River Laboratories Japan, Inc. The animals were 6 weeks old and 8 weeks old at the beginning of dosing.

【 Test substance 】

We used clofibrate (CAS No.: 637-07-0) that act through nuclear receptor.

【 Dose levels and treatment 】

Clofibrate was administered orally to the rats once a day for 28 consecutive days. For control, the vehicle was administered in the same way as clofibrate. Clofibrate was administered to the animals at 125 and 500 mg/kg/day.

【 Results and discussions 】

In animals aged 6 weeks at the start of dosing, a significant increase in liver micronucleus induction was observed in the 125 and 500 mg/kg/day groups. In animals aged 8 weeks at the start of dosing, although a significant increase in liver micronucleus induction was observed in the 500 mg/kg/day group, no significant increase was observed in the 125 mg/kg/day group. As for clofibrate used in this study, it was possible to detect liver micronucleus induction even in animals aged 8 weeks at the start of dosing. However, liver micronucleus induction was lower in 8-week-old animals at the start of dosing compared to 6-week-old animals at the start of dosing receiving the same dose of clofibrate. We believe that further examination is necessary to identify usefulness of 8-week-old animals at the start of dosing in detecting hepatocarcinogens in a 28-day repeated liver micronucleus assay and the difference in detection capability compared to 6-week-old animals at the start of dosing.

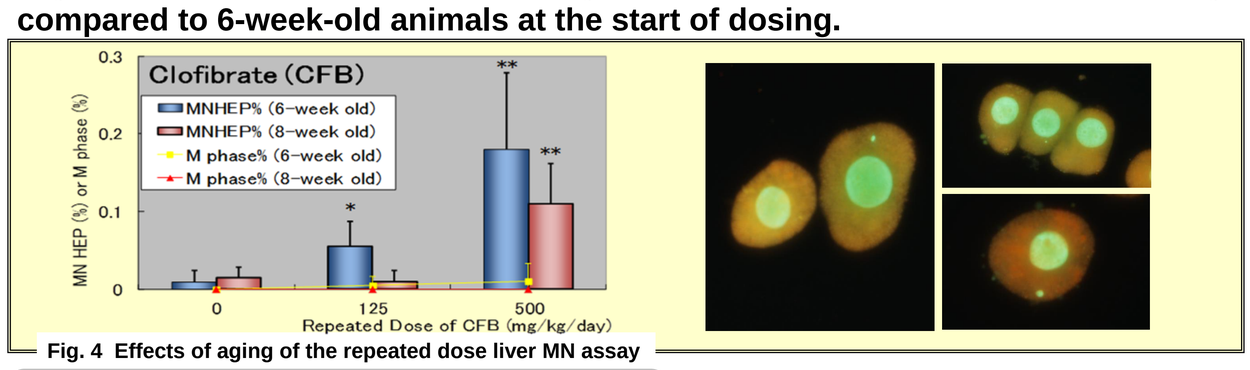

Fig. 4 Effects of aging of the repeated dose liver MN assay

Summary

1. **The working group members of 7th IWGT agreed that the liver MN assay is sufficiently validated in terms of numbers and types of chemicals studied to develop this test into an OECD Test Guideline.**
2. **This recommendation therefore supports the use of 6-week old rats, the age that was used to develop the majority of one month repeated dose liver MN assays have been accomplished to date. However, given the tendency of industry to conduct their general toxicology studies with rats older than 6 weeks at start of treatment, the impact of age at the time of dosing warrants further study.**
3. **In this study, we used clofibrate, a weak inducer of the liver MN, to compare the age difference between 6 and 8 weeks at the start of administration to examine effects of aging on the results of liver MN assays.**
4. **As for clofibrate used in this study, it was possible to detect liver micronucleus induction even in animals aged 8 weeks at the start of dosing.**
5. **We believe that further examination is necessary to identify usefulness of 8-week-old animals at the start of dosing in detecting hepatocarcinogens in a 28-day repeated liver micronucleus assay.**