Environ. Mutagen Res., 22 : 15 - 18(2000) Original Article

A micronucleus study of 2-methyl aziridine (propyleneimine) in rat peripheral blood and bone marrow by 4-week repeated exposure

Tomonori Aoki[1], Norihide Asano[2], Atsumune Imaeda[1] and Yasushi Kondo[1]

[1]Safety Research Laboratory, Tanabe Seiyaku Co., Ltd., 16-89 Kashima 3-Chome, Yodogawa-ku, Osaka 532-0031, Japan
[2]Biomedical Research Laboratory, Nitto Denko Corporation, 1-1-2 Shimohozumi, Ibaraki, Osaka 567-8680, Japan

Summary

Micronucleus induction in rat peripheral blood and bone marrow cells was studied after a 4-week repeated exposure to 2-methyl aziridine (2MA). 2MA was administered daily at 0, 2.5, 5, 10, and 20 mg/kg/day by oral gavage for 28 consecutive days. The peripheral blood samples were prepared on days 2, 3, 7, 14, 21, and 28 after the first administration, and bone marrow samples were prepared at 24 hours after the last administration. 2MA significantly induced micronucleated reticulocytes (MNRET) at 20 mg/kg/day and with two peaks at days 2 and 21. The time course and absolute values of the frequency of these MNRET were the same for micronucleated bone marrow cells. These findings therefore suggest that the micronucleus test with peripheral blood may be used as an alternative to the bone marrow micronucleus test in long-term toxicological assays in rats.

Keywords : micronucleus, bone marrow, peripheral blood, 4-week repeated exposure, 2-methyl aziridine

Introduction

Mice are widely used for detection of micronucleus in *in vivo*. The method in which the bone marrow samples are collected 24 hours after single dose is most widely employed in the micronucleus test. In general toxicity studies, on the other hand, such as single dose toxicity, repeated dose toxicity, and toxicokinetic studies, rats are used rather than mice. At our laboratory, repeated dose toxicity in rats by daily dosing for 2- or 4-week is routinely conducted to obtain preliminary toxicity information about the test compound in the early stage of drug discovery.

Recently, it is reported that the rat bone marrow and peripheral blood micronucleus assay can be used as well as the mouse micronucleus assay in short-term exposure (Wakata et al., 1998). However, the feasibility of rat bone marrow and peripheral blood micronucleus assay in long-term exposure is not well validated.

In the present study, the possibility of micronucleus induction in rats by 4-week repeated exposure was evaluated with 2-methyl aziridine. This study was performed as a part of the 13th Collaborative Study Group for the Micronucleus Test (CSGMT).

Materials and Methods

4-week-old male Slc : SD rats were purchased from Japan SLC Co., (Hamamatsu, Japan) and used for the experiments after a 1-week quarantine and acclimatization period. Commercial chow pellets in a basket and tap water were provided *ad libitum* throughout the periods of animal acclimatization and experimentation.

2-methyl aziridine (2MA, CASRN 75-55-8) was obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Dose levels for the test were based on a prior carcinogenicity study (Ulland et al., 1971).

received : December 6, 1999 accepted : February 28, 2000

2MA was diluted in distilled water, and administered at 0 (vehicle), 2.5, 5, 10 (5 animals per group), and 20 (7 animals per group) mg/kg/day. The test formulation of 10 mL/kg of body weight was administered to rats via the gastric tube orally daily for 28 days.

Body weight was measured weekly. Peripheral blood was collected from the ventraltail vessels on Days 2, 3, 7, 14, 21, and 28 after the first administration. Bone marrow smears were also prepared 24h after the last administration. Peripheral blood was diluted with an equal volume of fetal bovine serum and placed on an acridine orange (AO)-coated glass slide. 2000 reticulocytes (RET) per rat were examined for micronuclei by fluorescence microscopy (Hayashi et al., 1992). Bone marrow smears were stained with Giemsa. 1000 polychromatic erythrocytes (PCE) per rat were examined for micronuclei. The percentage of PCE (% PCE) versus all erythrocytes (PCE+NCE : normochromatic erythrocytes) was determined when the total number of PCE and NCE became 1000 or more.

The statistical analysis for the body weight change was conducted using t-test. For % PCE, data was analyzed by Dunnett test. And for the frequency of RET and PCE with micronuclei, the statistical significance of the differences between the vehicle group and exposed group were evaluated by the binominal test.

Results and Discussion

The highest dose, 20 mg/kg/day, was too toxic in the dose for the ordinary 4-week repeated exposure toxicity study because one of seven rats died on Day 18 and four of remaining six rats died on Day 24. And there was significant inhibition of body weight gain in the 20 mg/kg/day group on Days 7 and 21 compared with that of vehicle group (Fig. 1). The

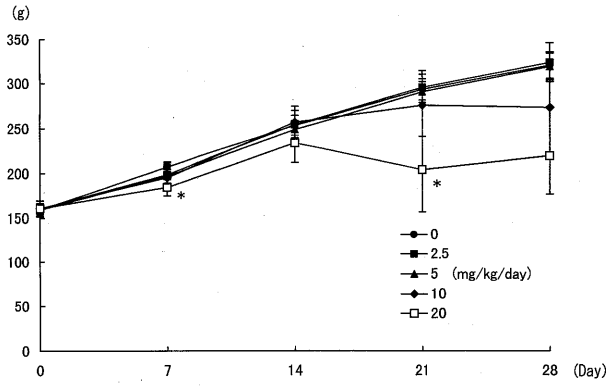

Fig. 1 Body weight change of 2MA-exposed rats for 4 weeks
Values are means±S.D.
0, 2.5, 5, 10 mg/kg/day : n=5
20 mg/kg/day : n=7 (Day 21 : n=6, Day28 : n=2)
* : Significantly different from vehicle control group at $p<0.05$ (t-test)
(The data of 20 mg/kg/day group on Day 28 was not analyzed)

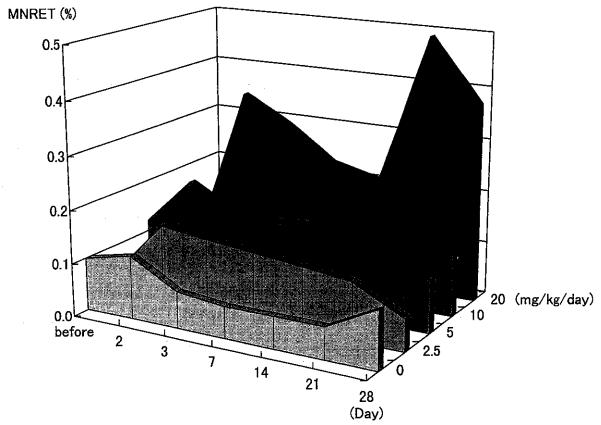

Fig. 2 Induction of MNRET in peripheral blood by 4-week repeated exposure of 2MA
Values are means
0, 2.5, 5, 10 mg/kg/day : n=5
20 mg/kg/day : n=7 (Day 21 : n=6, Day 28 : n=2)

Table 1 Induction of MNRET in peripheral blood by 4-week repeated exposure of 2MA

Treatment		No. of animals	% MNRET (mean±SD)						
			before	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28
Vehicle Control		5	0.10±0.07	0.12±0.10	0.07±0.03	0.06±0.05	0.06±0.20	0.06±0.07	0.11±0.10
2MA	2.5 mg/kg/day	5	0.05±0.04	0.15±0.11	0.14±0.08	0.13±0.10	0.12±0.12	0.11±0.08	0.06±0.02
	5 mg/kg/day	5	0.12±0.03	0.21±0.10	0.16±0.17	0.16±0.14	0.17±0.08*	0.12±0.22	0.10±0.11
	10 mg/kg/day	5	0.07±0.10	0.12±0.10	0.18±0.06	0.14±0.10	0.21±0.09**	0.11±0.17	0.16±0.05
	20 mg/kg/day	7	0.07±0.06	0.34±0.15**	0.29±0.16**	0.23±0.10**	0.21±0.09**	#0.49±0.23**	##0.38±0.25

Treatment : Compound and dosage
Vehicle : Distilled water
MNRET : Reticulocytes with micronuclei
: n=6 (1 of 7 rats died)
: n=2 (4 of 6 rats died)
Significantly different from vehicle control group * : $p<0.05$, ** : $p<0.01$ (binominal test)
(The data of 20 mg/kg/day group on Day 28 was not analyzed)

Table 2 Induction of MNPCE in bone marrow by 4-week repeated exposure of 2MA

Treatment		No. of animals	PCE observed	% MNPCE mean±SD	% MNPCE (range)	% PCE mean±SD	% PCE (range)
Vehicle Control	×28	5	10000	0.16±0.05	(0.10−0.20)	43.39±2.54	(39.85−46.20)
2MA 2.5 mg/kg/day	×28	5	10000	0.14±0.09	(0.05−0.25)	45.60±5.62	(38.40−51.19)
5 mg/kg/day	×28	5	10000	0.16±0.08	(0.10−0.30)	46.43±6.39	(37.86−53.42)
10 mg/kg/day	×28	5	10000	0.33±0.08	(0.25−0.45)*	35.48±4.06	(30.17−41.10)*
20 mg/kg/day	×28	#2	4000	0.38±0.11	(0.30−0.45)	36.00±1.41	(35.00−37.00)

Treatment : Compound, dosage, and multiplicity
Vehicle : Distilled water
PCE : Polychromatic erythrocytes
NCE : Normochromatic erythrocytes
MNPCE : PCE with micronuclei
% PCE : PCE/(PCE+NCE)×100
# : n=2 (5 of 7 rats died)
Significantly different from vehicle control group * : $p<0.05$ (% MNPCE : binominal test, % PCE : Dunnett test)
(The data of 20 mg/kg/day group was not analyzed)

results of the peripheral blood assay are shown in Table 1 and Fig. 2. The frequencies of micronucleated reticulocytes (MNRET) were significantly increased on any sampling days from Day 2 to Day 21 in the 20 mg/kg/day group and on Day 14 in the 5 and 10 mg/kg/day group. But the frequency of MNRET did not show clear serial changes on repeated exposure. The results of the bone marrow assay are shown in Table 2. The percentages of PCE versus all erythrocytes in the 2.5 and 5 mg/kg/day group were similar to that in the vehicle control group. Meanwhile the 10 mg/kg/day group was significantly lower than that in the vehicle control group as well as the 20 mg/kg/day group. The frequency of micronucleated polychromatic erythrocytes (MNPCE) in the 10 mg/kg/day groups was significantly higher (0.33 %) than that in the vehicle control group (0.16 %) and the 20 mg/kg/day group also showed high frequency (0.38 %). These results suggest that it is possible to use rat peripheral blood and bone marrow in 4-week repeated exposure study for the assessment of micronuclei.

2MA has been classified in group 2B (probable human carcinogen) by IARC (1975). 2MA was carcinogen in rats following oral administration producing a variety of malignant tumors (Ulland et al., 1971). Genotoxicity studies have also revealed the ability to induce sister chromatid exchanges in the Bloom syndrome B-lymphoblastoid cell line *in vitro* (Shiraishi, 1986) and mutagenic to bacteria (Dunkel et al., 1984). Furthermore, the positive results of the mouse (Morita et al., 1997) and the rat micronucleus assays (Wakata et al., 1998) have been shown by short term exposure.

In rats, it is reported that the frequencies of MNRET in peripheral blood and MNPCE in bone marrow by oral administration at 20 mg/kg of 2MA were about 0.2 % and 0.5 %, respectively. Our data of 4-week exposure presented here were similar to their results at the same dosage of 2MA. Asanami et al. (1995, 1996) and Henderson et al. (1993) showed increase in the incidences of MNRET and MNPCE by subchronic exposure in some compounds. In this study, however, the peak of micronucleus induction in 2MA was observed at Day 2 and 21 after the first administration, and the incidence of micronucleus induction reached a plateau during the period of repeated exposure. There is some possibility that the resemblance in peripheral blood assay was caused by rat spleen which captures and destroys circulating micronucleated erythrocytes (Schlegel and MacGregor, 1984), and accumulation of MNRET did not occur in peripheral blood. However, it is still difficult to interpret the reason for the difference in the pattern of time-course micronucleus induction among the clastogens.

In conclusion, the micronucleus assay using peripheral blood and bone marrow from rats in long-term exposure study is possible. Further studies using many chemicals are needed, however, to integrate the micronucleus assay into a 4-week toxicological assay for rats.

References

Asanami, S., K. Shimono, O. Sawamoto, K. Kurisu and M. Uejima (1995) The suitability of rat peripheral blood in subchronic studies for the micronucleus assay, Mutat. Res., 347, 73-78.

Asanami, S. and K. Shimono (1996) Detection of micronuclei in rat peripheral blood following oral administration of benzene for 14 days, MMS commun., 4, 1-5.

Director, A. E., J. Nath, M. J. Ramsey, R. R. Swiger and J. D. Tucker (1996) Cytogenetic analysis of mice chronically

fed the food mutagen 2-amino-1-methyl-6-phenylimidazo [4, 5*b*] pyridine, Mutat. Res., 359, 53-61.

Dunkel, V. C., E. Zeiger, D. Brusick, E. McCoy, D. McGregor, K. Mortelmans, H. S. Rosenkranz and V. F. Simmon (1984) Reproducibility of mutagenicity assay. I. Test with *Salmonella typhimurium* and *Escherichia coli* using a standard protocol, Environ. Mutagen., 6, Suppl. 2, 1-254.

Hayashi, M., Y. Kodama, T. Awogi, T. Suzuki, A. O. Asita and T. Sofuni (1992) The micronucleus assay using peripheral blood reticulocytes from mitomycin C-and cyclophosphamide-treated rats, Mutat. Res., 278, 209-213.

Henderson, L., J. Fedyk., S. Windebank and M. Smith (1993) Induction of micronuclei in rat bone marrow and peripheral blood following acute and subchronic administration of azathioprine, Mutat. Res., 291, 79-85.

ICRC (1975) ICRC monographs on the evaluation of carcinogenic risk to man, Vol. 9, Some azilidine, *N*-, *S*- & *O*-mustards and selenium, International Agency for Research on Cancer, Lyon (France).

MacGregor, J. T., J. D. Tucker, D. A. Eastmond and A. J. Wyrobek (1995) Integration of cytogenic assays with toxicology studies, Environ. Mol. Mutagen, 25, 328-337.

Morita, T., N. Asano, T. Awogi, YF. Sasaki, S. Sato, H. Shimada, S. Sutou, T. Suzuki, A. Wakata, T. Sofuni and M. Hayashi (1997) Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS. Collaborative Study of the Micronucleus Group Test. Mammalian Mutagenicity Study Group, Mutat. Res., 389(1), 3-122.

Kondo, Y., E. Nakajima, S. Nito, Y. Asano, F. Ariyuki and T. Higashiguchi (1994) Assessment of the human carcinogens arsenic acids and arsenic trichloride in the mouse micronucleus assay, MMS commun., 2, 117-121.

Jauhar, P. P., P. R. Henika, J. T. MacGregor, C. M. Wehr, M. D. Shelby, S. A. Murphy and B. H. Margolin (1988) 1, 3-Butadine : induction of micronucleated erythrocytes in the peripheral blood of B6C3F$_1$ mice exposed by inhalation for 13 weeks, Mutat. Res., 209, 171-176.

Tice, R. R., R. Boucher, C. A. Luke and M. D. Shelby (1987) Comparative cytogenetic analysis of bone marrow damage induced in male B6C3F$_1$ mice by multiple exposures to gaseous 1, 3-Butadine, Environ. Mutagen, 9, 235-250.

Schlegel, R. and J. T. MacGregor (1982) The persistence of micronuclei in peripheral blood erythrocytes : detection of chronic chromosome breakage in mice, Mutat. Res., 104, 367-369.

Schlegel, R. and J. T. MacGregor (1984) The persistence of micronucleated erythrocytes in the peripheral circulation of normal and splenectmized Fischer 344 rats : Implications for cytogenetic screening, Mutat. Res., 127, 169-174.

Schlegel, R., J. T. MacGregor and R. B. Everson (1986) Assessment of cytogenic damage by quantitation of micronuclei in human peripheral blood erythrocytes, Cancer Res., 46, 3717-3721.

Shiraishi, Y.(1986) Hypersensitive character of Bloom syndrome B-lymphoblastoid cell lines usable sensitive carcinogen detection, Mutat. Res., 175, 179-187.

Ulland, B., M. Finkelstein, E. K. Weisburger, J. M. Rice and J. H. Weisburger (1971) Carcinogenicity of industrial chemicals propylene imine and propane sultone, Nature, 230, 460-461.

Wakata, A., Y. Miyamae, S. Sato, T. Suzuki, T. Morita, N. Asano, T. Awogi, K. Kondo and M. Hayashi (1998) Evaluation of the rat micronucleus test with bone marrow and peripheral blood : Summary of the 9th collaborative study by CSGMT/JEMS・MMS, Environ. Mol. Mutagen, 32, 84-100.