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INFORMATION SECTION

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Effects of benzene in a micronucleus test on peripheral blood utilizing acridine orange-coated slides

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Summary

A micronucleus test was conducted on the peripheral blood of mice and rats utilizing acridine orange-coated slides (AO-coated method) after oral administration of benzene. Blood was sampled at 0, 24, 48, and 72 h after administration of benzene at doses of 500, 1000, and 2000 mg/kg in both mice and rats. The highest occurrence of micronucleated reticulocytes (MNRETs) was observed at 48 h after administration in both species. Species differences was found in the frequency of MNRETs, with the number being lower in rats than in mice. The present results indicate that the micronucleus test can easily detect chromosome aberrations in peripheral blood induced by benzene administration in both mice and rats. Furthermore, the AO-coated method used in this study was simpler to perform and allowed for easier detection of the effect than the conventional method.

The micronucleus assay on peripheral blood has the advantage that information can be obtained on chromosome aberrations in vivo without killing the animals. The advantage of performing the micronucleus test using acridine orange-coated slides (AO-coated method; Hayashi et al., 1990) was evaluated in the present study using the peripheral blood of benzene-treated mice and rats. This study was conducted as part of the fifth collaborative study by the Collaborative Study Group for the Micronucleus Test (CSGMT).

Materials and methods

Male Crj:CD-1 mice, 7 weeks of age, and male Crj:CD rats, 4 weeks of age, were purchased from Charles River Japan, Inc. (Atsugi, Japan). The mice and rats were used after 1 and 2 weeks of acclimatization, respectively. They were fed with commercial food pellets and tap water ad libitum throughout the acclimatization and experimental periods. The mean body weights $\pm$ standard deviations at the start of the experiment were 35.8 ± 1.4 g (mice) and 181.6 ± 8.3 g (rats). Benzene (CAS No. 71-43-2, Wako Pure Chemical Industries, Ltd., Osaka, Japan) was dissolved in olive oil. The doses of benzene were 500, 1000, and 2000 mg/kg, p.o., and were based on the results of the third collaborative study by the

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CSGMT (Suzuki et al., 1989). Peripheral blood was collected from the tail vein at 0, 24, 48, and 72 h after administration of benzene by gavage, and placed at the center of acridine orange-coated glass slides which had been prepared according to the method of Hayashi et al. (1990). After affixing coverglasses to the slides, they were left to stand overnight. The number of micronucleated reticulocytes (MNRETs) was then counted by observing 1000 reticulocytes (RETs) per animal by fluorescence microscopy, using a blue excitation filter and a yellow-to-orange barrier filter. The reticulocytes in the rats were identified by the same criteria as used in mice, and cells with only red fluorescing dot(s) were not regarded as reticulocytes in either species. Mitomycin C (MMC, Kyowa Hakko, Tokyo, Japan) was used as a positive control. The micronucleus test using mice was conducted at Preclinical Research Laboratories, Central Institute for Experimental Animals (CIEA) and at Pharmaceuticals Research Center, Toyobo Co., Ltd (Toyobo). The test using rats was also conducted at Toyobo.

Results and discussion

The mean frequency of MNRETs at 0 h after administration of benzene was $0.15 \pm 0.11\%$ (CIEA: $0.18 \pm 0.11\%$, Toyobo: $0.11 \pm 0.09\%$) in mice and $0.14 \pm 0.09\%$ in rats. In both mice and rats, micronuclei proved to be easier to score in the reticulocytes than the polychromatic erythrocytes that are targeted by staining with Giemsa.

The results of administering benzene to mice at CIEA and at Toyobo are shown in Table 1. The frequency of MNRETs increased at 24 h following administration of benzene and peaked at 48 h after each dose in both laboratories. The frequency of MNRETs at 48 h after each dose was similar to the reported maximum frequency of micronucleated polychromatic erythrocytes (MNPCEs) in bone marrow at 24 h after the administration of benzene (Suzuki et al., 1989). At 72 h after each dose, the frequency of RETs was lower; indeed, no RETs were found in 1 case of the 2000 mg/kg group at CIEA. It has been reported that the frequency of MNPCEs in bone

TABLE 1

MEAN FREQUENCY OF MNRETs $\pm$ SD FROM PERIPHERAL BLOOD IN MICE AFTER ORAL ADMINISTRATION OF BENZENE

Dose (mg/kg)	Testing facility ^a	Number of animals	Frequency of MNRETs based on 1000 RETs observed per animal (%)			
			0 h	24 h	48 h	72 h
Olive oil	A	5	0.16 ± 0.09	0.20 ± 0.07	0.34 ± 0.26	0.32 ± 0.23
	B	5	0.08 ± 0.08	0.14 ± 0.09	0.20 ± 0.10	0.14 ± 0.09
	Pooled	10	0.12 ± 0.09	0.17 ± 0.08	0.27 ± 0.20	0.23 ± 0.19
Benzene	500	A	0.18 ± 0.19	0.46 ± 0.05	1.60 ± 0.94	1.06 ± 1.49
		B	0.10 ± 0.07	0.38 ± 0.22	1.52 ± 0.89	0.72 ± 0.29
		Pooled	0.14 ± 0.14	0.42 ± 0.15	1.56 ± 0.86	0.89 ± 1.03
	1000	A	0.18 ± 0.11	0.72 ± 0.38	3.36 ± 3.37	1.16 ± 1.43
		B	0.12 ± 0.13	0.58 ± 0.19	2.14 ± 0.55	0.58 ± 0.20
		Pooled	0.15 ± 0.12	0.65 ± 0.29	2.75 ± 2.36	0.87 ± 1.01
2000	A	5	0.18 ± 0.04	0.48 ± 0.22	2.06 ± 0.52	0.68 ± 0.76
	B	5	0.12 ± 0.08	0.26 ± 0.15	3.44 ± 0.99	1.12 ± 0.40
	Pooled	10	0.15 ± 0.07	0.37 ± 0.21	2.75 ± 1.04	0.90 ± 0.62
MMC	1	A	0.20 ± 0.10	1.16 ± 0.33	3.16 ± 1.54	0.74 ± 0.42
		B	0.14 ± 0.11	0.64 ± 0.09	3.78 ± 0.84	1.04 ± 0.15
		Pooled	0.17 ± 0.11	0.90 ± 0.36	3.47 ± 1.22	0.89 ± 0.33

^a A, Preclinical Research Laboratories, CIEA; B, Pharmaceuticals Research Center, Toyobo Co., Ltd., Toyobo.

TABLE 2

MEAN FREQUENCY OF MNRETs $\pm$ SD FROM PERIPHERAL BLOOD IN RATS AFTER ORAL ADMINISTRATION OF BENZENE

Dose (mg/kg)	Number of animals	Frequency of MNRETs based on 1000 RETs observed per animal (%)			
		0 h	24 h	48 h	72 h
Olive oil	5	0.20 $\pm$ 0.07	0.22 $\pm$ 0.16	0.26 $\pm$ 0.15	0.22 $\pm$ 0.18
Benzene					
500	5	0.12 $\pm$ 0.11	0.24 $\pm$ 0.11	0.46 $\pm$ 0.15	0.24 $\pm$ 0.15
1000	5	0.14 $\pm$ 0.09	0.20 $\pm$ 0.07	1.00 $\pm$ 0.41	0.42 $\pm$ 0.08
2000	5	0.14 $\pm$ 0.09	0.16 $\pm$ 0.09	1.10 $\pm$ 0.27	0.48 $\pm$ 0.22
MMC					
1	5	0.12 $\pm$ 0.08	0.24 $\pm$ 0.21	1.38 $\pm$ 0.31	0.38 $\pm$ 0.11

marrow reached a peak at 24 h after administration of benzene (Suzuki et al., 1989), thus it seems that about 24 h is required for the transition from MNPCEs in bone marrow to MNRETs in the peripheral blood. The frequency of MNRETs at 48 h increased dose-dependently at Toyobo. However, no dose dependence at 48 h was observed at CIEA, where the highest frequency was observed at 1000 mg/kg. This may have been due to the influence of benzene appearing more strongly than at Toyobo, and to more extreme inter-individual differences than at Toyobo as noted with the very high frequency of MNRETs observed in 1 case of the 1000 mg/kg group at CIEA. It has been reported that the frequency of micronucleated normochromatic erythrocytes in mice treated with benzene for 14 days decreased from day 11 post administration (Rithidech et al., 1988).

The results after administration in rats are shown in Table 2. The time course of increase of MNRETs in the peripheral blood following benzene administration was like that in mice. However, the frequency of MNRETs in rats was lower than in mice. In addition, the frequency of MNRETs after administration of the positive control MMC was also lower in rats, although the frequency peaked at 48 h after administration in both species. This difference in MNRET frequency may be due to the difference in benzene metabolism in mice and rats (Medinsky et al., 1989; Sabourin et al., 1989) and also to loss of the MNRETs in the spleen in rats (Schlegel and

MacGregor, 1984). From the above results, it appears that chromosome aberrations induced by benzene can be easily detected in both mice and rats using peripheral blood rather than bone marrow for the micronucleus test. Furthermore, the AO-coated method used in the present study is simpler and permits easier observation of effects than more conventional methods.

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