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Rapid Communication

Histopathologic Findings of Bone Marrow Induced by 2-Bromopropane in Male Rats

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2-Bromopropane may have potential genital and hematopoietic toxicity in humans. This solvent is used as an alternative of chlorofluorocarbons. From the recent report in Korea[1], of 33 workers (8 men and 25 women) who were working in the production of electronic machine components (Tact Switch), using 2-bromopropane, 16 people (64%) had amenorrhea, two had azoospermia and four had oligozoospermia or reduced sperm motility; eight women and one men also had pancytopenia. These disorders were not seen among workers who were not exposed to the 2-bromopropane in the same factory. In addition, the onset of amenorrhea was 4–16 months after replacement of 1.1.2-trichloro-1.2.2-trifluoroethane with 2-bromopropane.

Ichihara *et al.*[2,3] confirmed the testicular toxicity of 2-bromopropane in animals by inhalation exposure ranging between 0–3,000 ppm: the chemical-induced damage occurred at 300 ppm or higher in male Wistar rats. Disruption of ovarian cyclicity induced by this solvent was also confirmed in female Wistar rats[4]. These results clearly demonstrate the genital toxicity of 2-bromopropane. However, hematotoxicity of 2-bromopropane has not been thoroughly investigated: only effects on peripheral blood cells were documented[2,3].

This study was done in an attempt to clarify the effect of 2-bromopropane on bone marrow in male Wistar rats, and the results were compared with those in genital tissues.

Material and Methods

2-Bromopropane (purity, over 99%) was kindly provided by Tosoh Co., Japan. Wright and Giemsa reagents were purchased from Merck (Darmsradt, Germany). Other chemicals used were purchased from Wako Chemicals (Osaka, Japan).

Thirty six male Wistar rats of nine weeks old were purchased from Nippon SLC Co., Ltd. (Shizuoka, Japan). The rats were housed in stainless steel cages with conditions at 22–25°C and 57–60% humidity for four weeks. Then, the rats were grouped into four, three were exposed to 2-bromopropane at 0 ppm-, 300 ppm- and 1,000 ppm-8 hr per day for nine weeks, respectively, by the method reported elsewhere[3,5]. The remaining group was also exposed to the chemical at 3,000 ppm-8 hr per day, but the exposure was from 9–11 days after the start, because all rats seemed to be seriously ill after the exposure. Three rats were killed 16–17 hr after cessation of the last exposure, and the remaining six were exposed to fresh air, similar to the control group.

All the rats were anesthetized with pentobarbital sodium 16–17 hr after cessation of the last exposure, and blood from the abdominal artery was withdrawn into heparinized syringes. Bone marrow was extracted from the left femur and was fixed in 10% buffered formalin, decalcified, and embedded in paraffin. The sections were stained with hematoxylin-eosin (HE) to investigate the cellularity and the number of megakaryocytes. Bone marrow (right femur) was smeared on a slide, and stained by Wright-Giemsa, as controls.

Semiquantitative analysis of bone marrow damage induced by 2-bromopropane was investigated by measuring the number of adipose cells and megakaryocytes on HE stained biopsy samples, and morphologic findings of the bone marrow cells were evaluated using Wright-Giemsa stained samples. The numbers of adipose cells and megakaryocytes were counted in an area (0.185 mm^2) of five sections per rat, under light microscopy and results were expressed by mean numbers per 1 mm^2. The numbers of granulocytes and erythrocytes were counted within 500 blood cells. Bone marrow cellularity was determined on the histological slide of Wright-Giemsa staining, and designated as a normocellularity when the ratio of adipose cells to hematopoietic cells was 1:2, a hypocellularity and a hypercellularity when the ratio was greater or smaller than that of normocellularity, respectively.

Analysis of variance was performed. Tukey-Kramer's multiple comparison method was used under the null-hypothesis that there is no significant difference between groups. The 0.05 level of probability was the criterion of significance.

Results

In control rats, numbers of adipose cells were 174 ± 77 / mm^2 (Table 1), a value similar to those of the group exposed to 300 ppm 2-bromopropane: the exposure to 300 ppm 2-bromopropane did not influence numbers in the bone marrow. On the other hand, exposure to 2-bromopropane at 1,000 ppm or higher dose-dependently increased the numbers of adipose cells accompanied by the decrease in hematopoietic cells. In the group exposed to 1,000 ppm 2-

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Table 1. Effects of exposure to 2-bromopropane on the number of megakaryocytes and adipose cells in rat bone marrow

2-bromopropane (ppm)	n	No. of megakaryocytes (/mm^2)	No. of adipose cells (/mm^2)
0	9	74 ± 17	174 ± 77
300	9	66 ± 16	186 ± 66
1000	9	39 ± 12[c,d]	479 ± 86[c,d]
3000	3[a]	21 ± 13[c,d]	1166 ± 224[c,d,e]
	(6)[b]	(51 ± 11[c])	(629 ± 99[c,d,e,f])

[a]Rats were exposed to 2-bromopropane for 11 days, and then killed 16–17 hr after the last exposure. [b]Rats were exposed to 2-bromopropane for 9–10 days and then exposed to fresh air for 7.5 weeks. [c]Significantly different from control ($p<0.05$). [d]Significantly different from group exposed to 300 ppm of 2-bromopropane. [e]Significantly different from group exposed to 1,000 ppm of 2-bromopropane. [f]Significantly different from group exposed to 3,000 ppm of 2-bromopropane for 11 days, and then killed 16–17 hr after the last exposure.

bromopropane, the numbers increased to 2.8-fold over those of control group. Although the duration of exposure was only one sixth in the group exposed to 3,000 ppm 2-bromopropane, the numbers of adipose cells were 7-fold higher than those of control rats. The cessation of exposure to 3,000 ppm 2-bromopropane for more than seven weeks led to a decrease in the numbers of adipose cells when compared to those in the group killed immediately after the exposure, but not to the level of 1,000 ppm- or lower-exposed groups. In addition, this cessation seemed to diminish the size of adipose cells (data not shown).

Exposure to 300 ppm 2-bromopropane did not influence the number of megakaryocytes, but exposure to 1,000 ppm or higher did decrease the numbers. The cessation of exposure to 3,000 ppm 2-bromopropane for more than seven weeks offset the numbers, but not to the control level.

The ratio of granulocytes to erythrocytes in control group was 1.88 ± 0.72, which was almost the same to that of the group exposed to 300 ppm 2-bromopropane (the ratio of granulocytes to erythrocytes, 1.79 ± 0.54). Exposure to 1,000 ppm 2-bromopropane or higher increased adipose cells with a few bone marrow cells, but not the ratio of granulocytes to erythrocytes (1.77 ± 0.65 and 1.77 ± 0.65, respectively). It is noticed that the size of adipose cells in 3,000 ppm exposed group was smaller than that in 1,000 ppm (data not shown), in good agreement with the result from HE stained sample. Residual bone marrow cells showed morphologically minimal change in each lineage.

Histopathologically, bone marrow from control rats showed normocellularity (6 of 9 samples) or slight hypercellularity (3 of 9), and that from 300 ppm 2-bromopropane-exposed rats normocellularity (4 of 9), slight hypercellularity (2 of 9) or slight hypocellularity (2 of 9). All samples of bone marrow from 1,000 ppm 2-bromopropane-exposed rats slightly showed hypocellularity, whereas that from the highest 2-bromopropane-exposed rats clearly showed hypocellularity.

Discussion

We reported that 2-bromopropane caused genital organ damage in male rats[2,3]. In the present study, exposure to 2-bromopropane led to a hypocellular bone marrow: there might be a decrease in numbers of hematopoietic cells, and the thus induced bone marrow hypoplasia might develop pancytopenia. These results clearly indicate that 2-bromopropane has the potential for genital organ and hematotoxicity. The pattern of anemia may be a slightly macrocytic[3]. These results also support the hypothesis that oligozoospermia and anemia observed in humans who work with electronic machine components in Korea[1] resulted from the exposure to 2-bromopropane.

2-Bromopropane at 300 ppm or higher induced testicular damage. In contrast, hematotoxicity caused by this solvent was not seen at this concentration. Thereby the testicles are more vulnerable to 2-bromopropane than is the bone marrow. Similar findings were also seen in workers in Korea[1]; oligozoospermia or amenorrhea occurred in 70% of the workers, but hematotoxicity only in 23%; all workers who had anemia also had amenorrhea or oligozoospermia, but no worker had anemia alone.

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