2-Bromopropane-Induced Hypoplasia of Bone Marrow in Male Rats

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Abstract: 2-Bromopropane-Induced Hypoplasia of Bone Marrow in Male Rats: Tamie NAKAJIMA, et al. Department of Hygiene, Shinshu University School of Medicine—The hematotoxicity of 2-bromopropane was investigated in thirty-six male Wistar rats. The rats were put into four groups: three groups were exposed to 0, 300, and 1,000 ppm 2-bromopropane for 8 hr per day, for 9 weeks, respectively, and the remaining group was exposed to 3,000 ppm only for 9-11 days. Hematotoxicity was assessed by measuring peripheral blood cells as well as cellularity, the number of megakaryocytes and morphological findings in the bone marrow. Exposure to 2-bromopropane decreased the numbers of erythrocytes in the peripheral blood at 300 ppm or higher, leukocytes at 1,000 ppm, and platelets at 300 and 1,000 ppm. Exposure to 300 ppm 2-bromopropane did not influence the indices of bone marrow toxicity. Exposure to 1,000 ppm 2-bromopropane or a higher dose-dependently induced a hypoplastic profile with replacement of fatty spaces in the bone marrow, though the durations of exposure to 3,000 ppm were under one sixth. These exposures also induced dose-dependent decreases in the number of megakaryocytes, with the maintained ratio of granulocytes to erythrocytes in the bone marrow. Residual progenitor cells showed some dysplastic or megaloblastic changes. These results suggest that exposure to 2-bromopropane leads to a reduction in the numbers of hematopoietic cells in the bone marrow, the result being a persistent pancytopenia in male rats.

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2-Bromopropane may have potential genital and hematopoietic toxicity in humans. This solvent is an alternative one replacing chlorofluorocarbons. From a recent report in Korea¹, 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 man also had pancytopenia. These disorders were not seen among workers who were not exposed to the 2-bromopropane. 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. The testicular toxicity was also confirmed in another laboratory, where 2-bromopropane was ingested intraperitoneally⁴. Recently, disruption of ovarian cyclicity induced by this solvent was confirmed in female rats⁵. These results clearly demonstrate the genital toxicity of 2-bromopropane, but the hematotoxicity of 2-bromopropane has not been thoroughly investigated: only its effects on peripheral blood cells were documented⁶.

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 findings in genital tissues.
Material and Methods

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

Animals: 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 put into four groups; three were exposed to 2-bromopropane at 0 ppm-, 300 ppm- and 1,000 ppm-8 hr per day for 9 weeks, respectively, by the method reported elsewhere3, 6). 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 the rats seemed to be seriously ill. Three rats were killed, and the left femur was dissected out 16-17 hr after cessation of the last exposure; the remaining six were exposed to fresh air, similarly to the control group.

All the rats excluding the three rats killed after 11 days' exposure were anesthetized with pentobarbitral sodium 16-17 hr after cessation of the last exposure, and blood from the abdominal artery was withdrawn into heparinized syringes to assess the blood cells. Bone marrow from all the rats 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.

Peripheral blood counts: The numbers of erythrocytes, leukocytes and platelets, hematocrit level, and concentration of hemoglobin in whole blood were measured with a Toa Microcell Counter (F-800, Toa Medical Electronics).

Analysis of bone marrow cellularity: Semi-quantitative analysis of bone marrow damage induced by 2-bromopropane was investigated by measuring the number of adipose cells and megakaryocytes, and the area of adipose cells on HE stained biopsy samples, and morphologic findings of the bone marrow cells were evaluated in Wright-Giemsa stained samples. The numbers of adipose cells and megakaryocytes were counted in an area (0.185 mm²) in five sections per rat, under a light microscope and the results were expressed as mean numbers per 1 mm². A field of these microscopic sections was also put into Adobe Photoshop 3.0.5J software (Adobe System, Tokyo) on a personal computer (Power Macintosh 8500, Apple Japan Inc., Tokyo), and the area of adipose cells was measured with NIH image 1.60/ppc software (National Institute of Health, USA). The results were expressed as percentages in a given microscopic field. 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.

Statistics: 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

Peripheral blood cell count

The time course of body weight of all the rats was reported previously3). The numbers of leukocytes, platelets and erythrocytes, the concentration of hemoglobin and level of hematocrit in the peripheral blood were significantly decreased (Fig. 1). A decrease in erythrocytes was seen with 300 ppm and higher of 2-bromopropane. Exposure to fresh air after exposure to 3,000 ppm for 9-11 days could not offset the numbers to those of the control. The total leukocyte count decreased only on exposure to 1,000 ppm 2-bromopropane, and the degree was most prominent (39%) among the indices of anemia. The number of platelets dose-dependently decreased at 300 and 1,000 ppm 2-bromopropane, and the degree (27%) at 1,000 ppm was between those for erythrocytes and leukocytes. The concentration of hemoglobin and the level of hematocrit decreased only at 1,000 ppm, but the degree was only marginal (4-5%).

Mean corpuscular volume and mean corpuscular hemoglobin values increased with exposure to 2-bromopropane of 300 ppm or higher, but that of the mean corpuscular hemoglobin concentration slightly decreased, only at the highest concentration, indicating that the anemia was slightly macrocytic.

Bone marrow cellularity

In control rats, numbers of adipose cells were 174 ±77/mm² (Fig. 2-a), values similar to those of the group exposed to 300 ppm 2-bromopropane (186 ±66/mm², Fig. 2-b): the exposure to 300 ppm 2-bromopropane did not influence numbers in the bone marrow. On the other hand, 1,000 ppm 2-
Fig. 1. Peripheral blood cell counts of rats exposed to 2-bromopropane (mean±SD for nine rats). MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. *Significantly different (p<0.05) from the rats exposed to 0 ppm of 2-bromopropane (Data in this figure have been reported elsewhere\textsuperscript{33}).
bromopropane 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-bromopropane, the numbers increased to 2.8-fold over those of control group (479±86/mm², Fig. 2-c). 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 (1166±224/mm², Fig. 2-d). 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 (629±99/mm²) when compared to those in the group killed immediately after the exposure, but not to a 1,000 ppm- or lower-exposed level. In addition, this cessation seemed to diminish the size of adipose cells (Fig. 2-e).

In control rats, the percentage of adipose cells in a given microscopic field of bone marrow was 14±6%, which was almost the same as that in the group exposed to 300 ppm 2-bromopropane (15±6%). In the group exposed to 1,000 ppm or more of 2-bromopropane, the percentage increased dose-dependently: the percentage was 41±8% in the 1,000 ppm group, and 68±6% in the 3,000 ppm group. The cessation of exposure to 3,000 ppm 2-
bromopropane for more than seven weeks led to a decrease in the percentage (41±3%) when compared to those in the group killed immediately after the exposure.

Exposure to 300 ppm 2-bromopropane did not influence the number of megakaryocytes (control group, 74±7/mm²; 300 ppm group, 66±16/mm²), but exposure to 1,000 ppm or higher did decrease the numbers (39±12/mm²). Exposure to 3,000 ppm also decreased the numbers (21±13/mm²). The cessation of exposure to 3,000 ppm 2-bromopropane for more than seven weeks offset the numbers, but not to the control level (51±11/mm²).

Figure 3-a shows findings in the case of Wright-Giemsa staining of bone marrow smears from control rats. Adipose cells scattered slightly. The ratio of granulocytes to erythrocytes in the control group was 1.88±0.72, which was almost the same to that in the group exposed to 300 ppm 2-bromopropane (Fig. 3-b; 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 (Fig. 3-c and 3-d; the ratios, 1.77±0.65 and 1.77±0.65, respectively). It was noticed that the size of the adipose cells in 3,000 ppm exposed group was smaller than that in the 1,000 ppm group, in good agreement with the result for the 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 showed slight 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 rats². In the present study,
exposure to 2-bromopropane led to hypocellular bone marrow: there might be a decrease in numbers of hematopoietic cells, and bone marrow hypoplasia the thus induced might develop pancytopenia. Similar results were also observed in females exposed to 2-bromopropane (unpublished data). These results clearly indicate that 2-bromopropane has the potential for genital organ toxicity and hematotoxicity. The pattern of anemia may be slightly macrocytic. These results also support the hypothesis that the oligozoospermia and anemia observed in humans who work in making electronic machine components in Korea 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. The testicles are therefore more vulnerable to 2-bromopropane than is the bone marrow. Similar findings were also seen in workers in Korean: oligozoospermia or amenorrhea occurred in 70% of the workers, but hematotoxicity in only 23%; all workers who had anemia also had amenorrhea or oligozoospermia, but no worker had anemia alone.

Some organic solvents that have the potential to cause aplastic anemia. Benzene\(^7\) is a good example, and ethylene glycol ethers\(^8\) are others. Both chemicals also have the potential to cause genital organ toxicity\(^9,11\). It would be interesting to know whether chemicals with hematotoxicity also have genital toxicity. Several halogenated propanes cause genital organ toxicity\(^12\), but little information about the hematotoxicity of these halogenated chemicals has been reported, and it should be investigated.

Potential mechanisms of 2-bromopropane responsible for the development of the bone marrow aplasia are 1) induced defects in hematopoietic stem cells, 2) failure of the stromal microenvironment of the bone marrow, or 3) impaired production of hematopoietic growth factors. Further investigations are needed to explain the mechanisms of hematopoietic toxicity induced by 2-bromopropane.

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References