Influence of 2-Bromopropane on Reproductive System — 2-Bromopropane Inhibits Forced Ovulation in Mice

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Abstract: The influence of 2-bromopropane, an alternative to freon, on ovulation was examined using superovulation induced by pregnant mare's serum gonadotropin and human chorionic gonadotropin (PMSG-hCG) treatment. Three groups of mice were injected intraperitoneally 8 times with 2-bromopropane (2-BP) at 500, 1,000 or 2,000 mg/kg and were induced to ovulate by PMSG-hCG treatment. Injection of 2-BP caused the number of ovulated ova in the 1,000 mg/kg and 2,000 mg/kg groups to decrease remarkably. Uterus weight was also decreased in the 2,000 mg/kg group on the terminal day of the experiment. This result was consistent with cases of human intoxication in which reproductive toxicity was observed.

Key words: 2-Bromopropane, Superovulation, PMSG, hCG, Mice

A case of mass intoxication has occurred in workers exposed to solvents containing 97.4% 2-bromopropane (2-BP) in an electronics factory at Yangsan in Korea¹⁾. In this case, 17 of 25 women and 6 of 9 men exhibited amenorrhea and oligozoospermia or azoospermia, respectively, and 9 of those 23 (17+6) workers also showed pancytopenia. Therefore, it was speculated that 2-BP might have toxic effects on reproductive and hematopoietic functions, although no such toxic effects of 2-BP were known before this incident.

Recently, several studies have clarified the effects of 2-BP on reproductive function, particularly on estrous cycles, in female rats^{2, 3)}. Estrous cycles were examined by means of a vaginal smear test in those studies. Lim *et al.* reported that, during a 2-week course of injection with 300 and 600 mg/kg of 2-BP, the duration of the estrous cycle did not obviously differ from that of control rats²⁾. However, in rats injected with a high dose (900 mg/kg) of 2-BP, the duration of the cycle significantly increased in accordance with the increment of the diestrus stage²⁾. The fertility index of 2-BP-treated rats declined with an increase in the dose of 2-BP²⁾. In a 9-week inhalation exposure study of 2-BP

reported by Kamijima *et al.*³⁾, the estrous cycle in rats exposed to 300 and 1,000 ppm of 2-BP was prolonged significantly. In histopathological examination, the number of viable follicles and corpora lutea in the ovaries was found to have decreased³⁾. These findings were thought to indicate that 2-BP affects the ovarian function, but they, did not directly show the decrease in the number of ovulated ova in 2-BP treated rats. Therefore, in the present study, we investigated the influence of 2-BP on ovulation in mice.

Experimental Animals and Design: Twenty female mice of ICR strain (Charles River Japan, Inc., Japan), aged 41–43 days, were caged under conditions of 12 h light (08:00 to 20:00)/12 h darkness. Room temperature and humidity were maintained at $23 \pm 1^\circ\text{C}$ and $55 \pm 5\%$, respectively. Feed and water were freely available. After the mice were kept in cages for 10 days under the above regimen, they were evenly distributed among 4 groups (5 mice/group) on the basis of body weight and were subjected to the intraperitoneal administration of 2-BP (Tokyo Chemical Industry, Co., Ltd., Japan) or its vehicle, olive oil (Wako Pure Chemical Industries, Ltd., Japan, 2 ml/kg). 2-BP was injected 8 times at intervals of 2 or 3 days for 17 days (Fig. 1). In 3 experimental groups, injection of 2-BP was conducted

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Table 1. Influence of 2-BP administration on body weight and liver weight

Dose (mg/kg)	0	500	1,000	2,000
Number of mice	5	5	5	4
Initial B.W. (g) ¹⁾	26.48 ± 1.01	26.44 ± 1.01	26.46 ± 0.95	26.76 ± 1.01
Terminal B.W. (g)	29.91 ± 1.79	30.44 ± 2.06	29.38 ± 1.40	27.53 ± 1.38
Liver weight (g)	1.685 ± 0.23	1.893 ± 0.10	1.775 ± 0.12	1.587 ± 0.16
Liver weight (%) ²⁾	5.618 ± 0.54	6.221 ± 0.24	6.058 ± 0.56	5.755 ± 0.31

Values are mean ± standard deviation. 1) B.W. indicates body weight. 2) Relative liver weights (%) are calculated as follows: [liver weight (g)] / [body weight (g)].

Fig. 1. Time-course changes of body weight in female mice

Vehicle only or 2-BP was administered at all points except the terminal point (day 18). * represents a statistically significant difference from the corresponding control at $P < 0.05$ based on Dunnett's multiple comparison.

↑ and ↑̂ indicate administration of PMSG and hCG, respectively.

at a dosage of 500, 1,000 or 2,000 mg/kg. Superovulation treatment was performed using pregnant mare's serum gonadotropin and human chorionic gonadotropin (PMSG and hCG, respectively, Teikoku Hormone MSG. Co., Ltd., Japan) as described by Jackson *et al.*⁴⁾. The two hormones were injected intraperitoneally at a dosage of 10 i.u./mouse at a 48 h interval on the 15th and 17th day of 2-BP injection, respectively (Fig. 1). All mice were sacrificed for autopsy the day after the final 2-BP injection, and their liver, uterus and oviduct were removed and examined. The liver and uterus were weighed, and the number of ovulated ova in

the oviduct were counted in each mouse.

Body weights did not differ among the mice on the initial day of the experiment (Table 1). One mouse in the 2,000 mg/kg group died on the 10th day of the experiment and this mouse was excluded from the subsequent statistical treatment. Fig. 1 shows time-course changes of body weight. There was no difference in the weight of mice between the 500 mg/kg and control groups throughout the experimental period. However, the body weight gain of 2-BP-treated mice was dose-dependently suppressed in the 1,000 and 2,000 mg/kg groups. On the terminal day of the experiment, body and liver weights in mice injected with 2-BP at 1,000 and 2,000 mg/kg were not significantly different from those in control mice (Table 1). Uterus weight decreased in the 2,000 mg/kg group, but did not decrease in the 500 mg/kg group or in the 1,000 mg/kg group. The mean number of ovulated ova in the 1,000 mg/kg group (23.75) was about half that in the control group (52.75), and the difference was statistically significant. In the 2,000 mg/kg group, the number of ova (6.00) further decreased compared with that in the 1,000 mg/kg group. Even in the 500 mg/kg group, the mean number of ova (44.80) was less than that in the control group, though the difference was not significant (Table 2). Kamijima *et al.*³⁾ showed that uterus weight decreased in rats exposed to 300 and 1,000 ppm of 2-BP. Moreover, their histopathological observation of ovaries revealed a decrease in viable follicles and corpora lutea, which strongly suggested that ovulation is suppressed in 2-BP treated rats³⁾. This may occur because 2-BP damages oocytes, and interferes with follicular maturation³⁾. The present results showing a decrease in uterus weight in the 2,000 mg/kg group and the failure of PMSG-hCG-induced ovulation in the 1,000 and 2,000 mg/kg groups are consistent with the results of Kamijima *et al.* On the other hand, Watanabe *et al.*⁵⁾ have reported that no estrus was induced in female rats in which the increase in body weight was interrupted under 2–4 hr/day feeding conditions. At present, we cannot completely exclude the possibility

Table 2. Influence of 2-BP administration on reproductive physiology

Dose (mg/kg)	0	500	1,000	2,000
Number of mice	5	5	5	4
Number of ova	52.25 ± 7.41	44.80 ± 9.52	23.75 ± 4.57*	6.00 ± 2.94*
Number of ova (%)	100	86	45	11
Uterus weight (g)	0.119 ± 0.02	0.139 ± 0.02	0.119 ± 0.04	0.082 ± 0.01
Uterus weight (%) ¹⁾	0.398 ± 0.06	0.455 ± 0.06	0.404 ± 0.14	0.294 ± 0.04

Values are mean ± standard deviation. * represents a statistically significant difference from the corresponding control at $P < 0.05$ based on Dunnett's multiple comparison test. 1) Relative uterus weights (%) are calculated as follows: [uterus weight (g)] / [body weight (g)].

that, particularly in the 2,000 mg/kg group, the decrease in body weight interfered with ovulation. However, the body weight in the 500 mg/kg group did not differ from that in the control group throughout the experimental period, and the number of ovulated ova in the former group was less than that in the latter group.

The present study showed that 2-BP treatment interrupted ovulation induced by PMSG-hCG in mice. Further study should be performed to investigate the mechanism of the inhibitory effect of 2-BP on ovulation.

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