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Comparative Testicular Toxicities of Two Isomers of Dichloropropanol, 2,3-Dichloro-1-propanol, and 1,3-Dichloro-2-propanol, and Their Metabolites Alpha-Chlorohydrin and Epichlorohydrin, and the Potent Testicular Toxicant 1,2-Dibromo-3-chloropropane

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Dichloropropanols are colorless liquids with an ethereal odor, and they are used extensively in many industrial processes (e.g. production of epichlorohydrin). Haratake et al. (1993) reported two cases of fulminant liver injury to workers who cleaned a dichloropropanol tank, and they detected two isomers of dichloropropanol, 2,3-dichloro-1-propanol (DC1P) and 1,3-dichloro-2-propanol (DC2P), in serums of one worker. They estimated from their experiment using rats that DC2P caused liver injury but DC1P did not cause such a injury. However, we reported that both DC1P and DC2P caused liver injury and kidney injury to mice (Hirata et al. 1993). Koga et al. (1992) reported that alpha-chlorohydrin (ACH) was formed in the metabolic pathway of DC1P and DC2P to 1,2-propanediol in rats. Epichlorohydrin (ECH) was formed during the course of transformation of DC2P to ACH in some bacteria (Nakamura et al. 1992) and the same transformation may occur in rats. ACH and ECH are well known to cause damages on the male reproductive system of experimental animals (Coppola 1969; Hahn 1970; Paz and Homonnai 1982; Kluwe et al. 1983; Toth et al. 1989). Therefore, it is possible that DC1P and DC2P have a testicular toxicity. However, there were no studies evaluating the testicular toxicity of these two isomers of dichloropropanol.

In this study, we investigated the comparative testicular toxicities of DC1P, DC2P, ACH, ECH, and 1,2-dibromo-3-chloropropane (DBCP) in rats. DBCP was a nematocide which was banned from use because this compound caused male infertility in human (Whorton 1977; Milby 1980). At six week after a single subcutaneous injection of each compound at a common dose of 0.34 mmol/kg body weight, the effects of these compounds on male reproductive system were evaluated using testicular and epididymal weight, homogenization-resistant spermatid count and sperm count, sperm morphology, and histopathology of the testis.

MATERIALS AND METHODS

Fifty-one male Wistar rats (Crj:Wistar), five weeks of age, were purchased from Charles River Japan Inc. (Yokohama, Japan). They were acclimated to the animal facility for seven weeks prior to the initiation of the experiment. At 12 weeks of age, a mean body weight of 392 g (340 - 467 g), the experiment was begun. The animals were housed four or five per cage in the SPF room of the Laboratory of Animal Experiments, Faculty of Medicine, Kyushu University. The light cycle was 12 hours:12 hours (light/dark), the temperature was 23 - 25 ℃, and the air humidity was 50 - 65%. The rats were provided with CE-2 (Clea Japan Inc.,

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Tokyo, Japan) and tap water *ad libitum*.

All rats were weighed and randomly distributed into five treatment groups plus one control group. Eight or nine animals were assigned to each group. Each compound was administered by a single subcutaneous injection at a common dose of 0.34 mmol/kg body weight (43.7 mg/kg for DC1P and DC2P, 37.4 mg/kg for ACH, 31.3 mg/kg for ECH, and 80 mg/kg for DBCP). This dosage was used according to the dosage of DBCP in the report of Kluwe et al. (1983). DBCP was dissolved in dimethylsulfoxide and other compounds were dissolved in saline just before administration. In the case of control group, rats received in the same manner, an identical volume of saline only.

At six week after the injection, the rats were sacrificed using an overdose of ether and the testes and the epididymes of both sides were removed and weighed. The right testis was fixed in Bouin's solution, embedded in paraffin, and stained with periodic acid Schiff reagent (PAS) and Mayer's hematoxylin for light microscopical examinations. The right epididymis was used to investigate sperm morphology. The tail of the epididymis was opened by a razor and the sperm was squeezed out into 0.1 M sodium phosphate buffer (pH 7.2). A smear was made on a glass slide coated with 2% neoprene solution in toluene and stained with Mayer's hematoxylin. For each smear, 1000 sperms were examined and the numbers of sperm with an immature head, sperm with a teratic head, and sperm without a tail were counted. Sperm with an immature head and that with a teratic head were classified according to the method of Mori et al. (1991). The left testis and epididymis were used for the sperm count. The testis was decapsulated and the epididymis was divided into two portions (head and body plus tail). Each part was homogenized in saline triton merthiolate solution (STM solution; 17.5 g of NaCl, 1 ml of triton X-100, and 0.2 g of sodium ethylmercurithiosalicylate were dissolved in distilled water for one litre of STM solution) with POLYTRON (KINEMATICA, Littau/Luzern, Switzerland) for 90 seconds. In the case of the testis and body plus tail of the epididymis, 200 ml of STM solution was used, while 90 ml of STM solution was used for the head of the epididymis. A hemocytometer was used for the counting of sperm. The examinations of sperm morphology and sperm count were performed by two experienced researchers, and the mean values which they obtained were used for our evaluations.

Data were analyzed for mean and standard deviation and compared by F test and then two sample t test. Test results were interpreted as significant below a level of 0.05.

All test reagents were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). The minimum purity of these reagents was as follows: DC1P 98%, DC2P 96%, ACH 98%, ECH 99%, DBCP 98%.

RESULTS AND DISCUSSION

Table 1 shows body weight, the testicular weight, and the epididymal weight of the rats at six week post-treatment. Organ weight refers to the summation of the weights of both sides per 100 g of body weight. Body weight in the DBCP group decreased significantly compared with that in the control group. The differences in body weight between other treatment groups and the control group were not significant. The testicular weight decreased significantly in the DBCP group only. The testicular weight in the DC1P and DC2P groups decreased, although the

Table 1. Effects of five compounds on body, testicular, and epididymal weights.

Compounds administered	Body weight (g) (mean(SD))	Testis (g) (mean(SD))	Epididymis (g) (mean(SD))
Control (n=9)	501.00(27.11)	0.757(0.067)	0.229(0.017)
DBCP (n=8)	434.63(34.57)**	0.373(0.027)***	0.156(0.015)***
ECH (n=8)	488.44(32.00)	0.787(0.089)	0.229(0.027)
ACH (n=9)	501.00(34.89)	0.788(0.076)	0.231(0.015)
DC1P (n=9)	510.22(35.00)	0.730(0.052)	0.208(0.014)*
DC2P (n=8)	499.88(31.94)	0.723(0.034)	0.220(0.016)

The organ weight refers to the summation of the weights of both sides, per 100 g body weight. Significantly different from control : *p<0.01, **p<0.0005, ***p<0.0001.

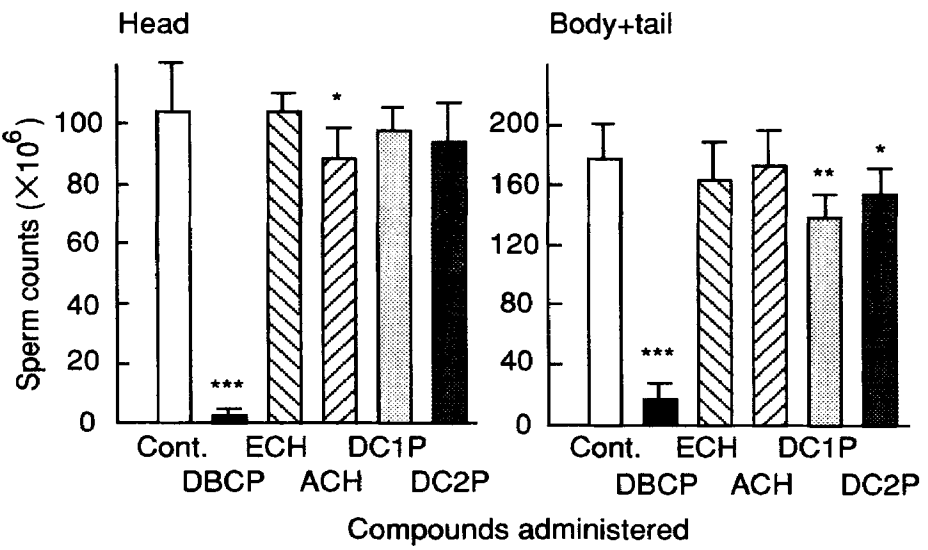

Figure 1. Effects of five compounds on sperm counts in the head and the body plus tail of the epididymis. All results are described as a mean ± SD (n=8 for the DBCP, ECH, and DC2P group; n=9 for the control, ACH, and DC1P group) . Significantly different from control : *p<0.05, **p<0.01, ***p<0.0001.

Table 2. Effects of five compounds on the rate of sperm with morphological abnormalities.

Compounds administered	Immature	Teratic	Without a tail
	(Abnormal sperm / 1000 sperm)		
Control (n=9)	3.8(2.3)	0.6(0.5)	13.8(9.2)
DBCP (n=8)	3.4(1.5)	0.6(0.5)	865.8(130.4)**
ECH (n=8)	3.3(2.4)	0.4(0.7)	14.7(6.2)
ACH (n=9)	7.3(4.1)*	1.6(1.4)	15.3(8.3)
DC1P (n=9)	4.4(4.6)	1.9(2.0)	25.0(21.5)
DC2P (n=8)	3.1(2.4)	0.5(0.8)	10.6(4.7)

Results are expressed as mean(SD). Significantly different from control : *$p<0.05$, **$p<0.0001$.

differences were not significant. The epididymal weight in the DBCP group decreased significantly. Moreover, the epididymal weight in the DC1P group decreased significantly as well.

Figure 1 compares the sperm count in the two portions (head and body plus tail) of the epididymis. In the DBCP group, the sperm count in both two portions decreased severely. The sperm count in the head of the epididymis was $103.9 \pm 15.8 \times 10^6$ in the control group and $0.3 \pm 0.4 \times 10^6$ in the DBCP group, and the sperm count in the body plus tail of the epididymis was $177.4 \pm 21.9 \times 10^6$ in the control group and $15.5 \pm 10.1 \times 10^6$ in the DBCP group. The sperm count in the DC1P and DC2P groups decreased significantly in the body plus tail of the epididymis. The sperm count in this portion was $138.9 \pm 13.4 \times 10^6$ in the DC1P group and $153.8 \pm 17.2 \times 10^6$ in the DC2P group. The sperm reduction in the DC1P group was severer than that in the DC2P group. The sperm reduction in the ACH group was also significant in the head of the epididymis. The count of homogenization-resistant spermatid in the testis decreased significantly in the DBCP group only (data not shown).

Table 2 shows the rate of sperm with morphological abnormalities. The rates of sperm with an immature head, sperm with a teratic head, and sperm without a tail were evaluated. In the DBCP group, the rate of sperm without a tail increased remarkably (13.8 ± 9.2 / 1000 in the control group and 865.8 ± 130.4 / 1000 in the DBCP group). The rate of sperm with an immature head increased slightly in the ACH group. In the DC1P, DC2P and ECH group, sperm with morphological abnormalities did not increase.

Figure 2A shows the stage IX seminiferous tubule in the control group. Step 9 spermatids, pachytene spermatocytes, and leptotene spermatocytes arranged regularly, and residual bodies and a few step 19 spermatids line the luminal surface of the epithelium. Figure 2B shows the seminiferous tubules in the DBCP group.

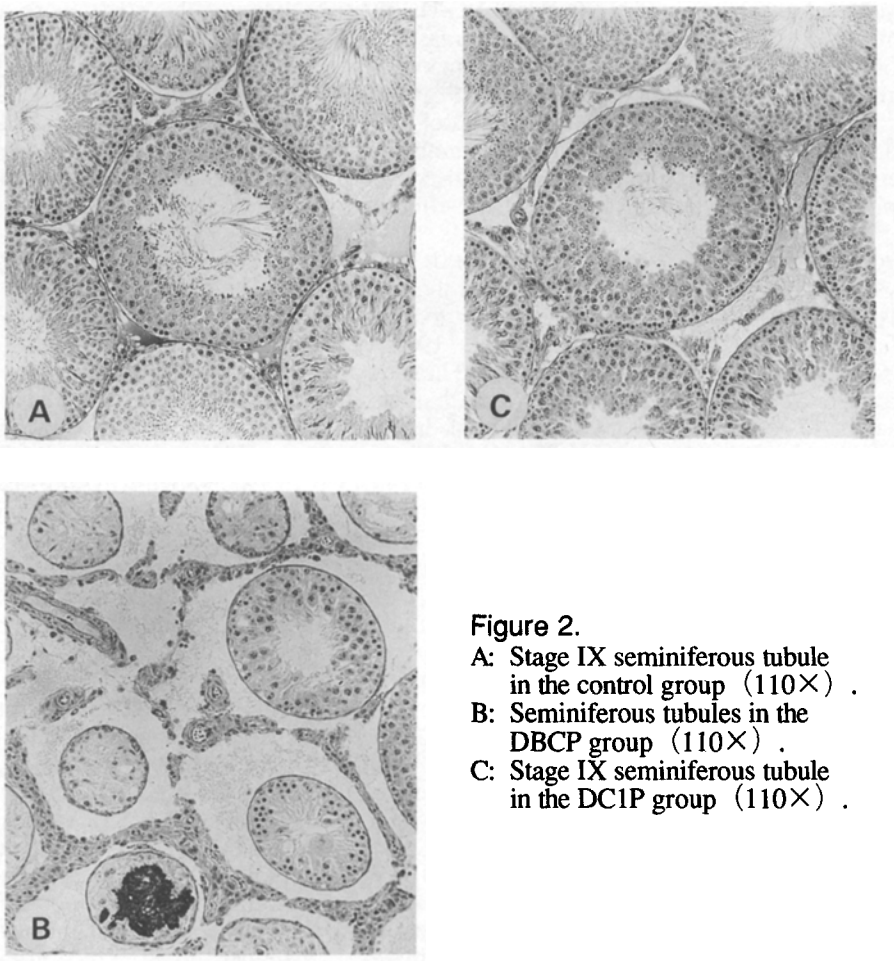

Figure 2.
A: Stage IX seminiferous tubule in the control group (110×).
B: Seminiferous tubules in the DBCP group (110×).
C: Stage IX seminiferous tubule in the DC1P group (110×).

All seminiferous tubules reduced their diameter remarkably. Most of step 9 spermatids disappeared in the stage IX tubule located at the upper right side of the figure, and even pachytene spermatocytes could scarcely be found in the tubule located at the lower right side. In the other tubules, only Sertoli cells could be seen. Figure 2C shows the stage IX seminiferous tubules in the DC1P group. Histological appearance is indistinguishable from that in the control group. No pathological changes were observed in seminiferous tubules in the DC2P, ACH, and ECH group, either.

Kluwe et al. (1983) administered DBCP, ACH, and ECH to male rats by a single subcutaneous injection and confirmed the toxic effect of these compounds on the male reproductive system at 25 and 75 day after the treatment. According to their method, we investigated the comparative testicular toxicity of DC1P, DC2P, and their metabolites ACH and ECH, and the potent testicular toxicant DBCP. These compounds were administered by a single subcutaneous injection at a common dose of 0.34 mmol/kg body weight and the comparative testicular toxicities were

evaluated at six week after the injection. The main findings of this study were as follows: (1) The epididymal weight decreased in the DC1P group, and the sperm count in the body plus tail of the epididymis decreased in both the DC1P and DC2P groups. The sperm reduction in the DC1P group was severer than that in the DC2P group; (2) DBCP caused severe atrophies of both the testis and the epididymis, and caused severe sperm reduction. The number of sperm without a tail increased remarkably in this group; (3) ACH caused sperm reduction in the head of the epididymis. ECH did not cause any toxic effects on male reproductive system.

From our findings, it was confirmed that DC1P is toxic to the rat male reproductive system. This is the first report indicating the testicular toxicity of DC1P. Our study also suggested that DC2P is a testicular toxicant in rats, but weaker than DC1P. Haratake et al. (1993) and Hirata et al. (1993) reported that the hepatotoxicity of DC1P is weaker than that of DC2P, and Hirata et al. (1993) reported that the kidney damage caused by DC1P is severer than that caused by DC2P. It is very interesting that the toxicities of these two isomers of dichloropropanol on these organs differ. Koga et al. (1992) proposed that DC1P produces ACH and 2-chloro-1,3-propanediol, and DC2P produces ACH only in their metabolic pathways in rats. The difference in their metabolic pathways may contribute to the difference in their toxicities on these organs. Koga et al. (1992) reported that the proposed pathways could explain only 1 - 14 % of the metabolic changes of these compounds and there may be other metabolic pathways besides the proposed one. Further studies are required to explain the difference in the organ toxicities of DC1P and DC2P.

To compare the testicular toxicities of DC1P, DC2P, ACH, ECH, and DBCP, we administered a equimolar dose of these compounds in this study. As a result, the testicular toxicity of DC1P was stronger than that of DC2P, ACH, and ECH, and was far weaker that that of DBCP when 0.34 mmol/kg body weight was applied as a dose administered. The severe toxic effects of DBCP on the male reproductive system was consistent with those previously reported (Kluwe et al. 1983). We also found that the rate of sperm, without a tail, increased in the body plus tail of the epididymis following administration with DBCP. The epididymis was squeezed to collect sperm for the evaluation of sperm morphology in this study. It is possible that some sperms in the DBCP group which originally had their tails might have lost them during the collection procedure. However, there was no difference in the sperm collection procedure between the DBCP group and the control group, and so the increase in the rate of sperm, without a tail, in the DBCP group can not be explained this way. The increase of such sperm probably reflects an increase in the fragility of the sperm neck caused by DBCP. The effects of ACH and ECH on the male reproductive system were very weak compared with those reported by Kluwe et al. (1983). This is probably due to low doses administered in this study. The dose of ACH was less than half and the dose of ECH was less than one-third compared with those in the study of Kluwe et al. (1983).

In conclusion, the testicular toxicities of two isomers of dichloropropanol, DC1P and DC2P, were observed in this study. The testicular toxicity of DC1P was stronger than that of DC2P, ACH, and ECH, and was far weaker than that of DBCP.

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