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原　　著

Dose-dependent Testicular Toxicity of Propylene Oxide in Rats Induced by Repeated Intraperitoneal Injections

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Abstract The dose-dependent testicular toxicity of propylene oxide (PO) was evaluated in male Wistar rats when administered by intraperitoneal injections. In 23 mg/kg, 47 mg/kg, and 93 mg/kg groups, PO was given three days a week for six weeks, while PO was given three days a week for two weeks plus once a week after the third week in a 186 mg/kg group. In the 186 mg/kg group, the epididymal weight and sperm count in the body plus tail of the epididymis decreased, while the rate of sperm with morphological abnormalities increased significantly. The number of sperm with an immature head increased slightly, although significantly, even in the 47 mg/kg group and a dose-dependent effect could be seen. The serum testosterone concentration did not change significantly and there were no apparent histopathological changes in Leydig cells in any of the treatment groups. This is the first detailed study concerning the dose-dependent testicular toxicity of PO.

Introduction

Propylene oxide (PO) is a volatile and highly reactive alkylating agent. PO is used extensively as an intermediate in the production of various chemicals (e.g. polyether polyol and propylene glycol) with further minor usage in the sterilization or fumigation of medical equipment and foodstuff. In previous studies[5)8)9)10)], we first found the definite testicular toxicity of ethylene oxide, an epoxide with the simplest chemical structure. As for PO, Antonova et al.[1)] reported that a single oral administration of a LD_{50} dose (520 mg/kg) of this substance slightly reduced the "motile time of sperm" (196 min. in the control group and 141 min. in the exposure group) and significantly reduced the fertility rate (100% in the control group and 52.2% in the exposure group) in rats. A decrease in testicular weight[6)13)] and a reduction in sperm count[7)] were also reported after exposure to this substance in animal experiments. However, there have been no previous studies extensively evaluating the testicular toxicity of PO.

In this study, we investigated the dose-dependent effects of PO on testicular and epididymal weight, sperm count, sperm morphology, histopathology of the testis, and serum concentration of testosterone in rats, when injected intraperitoneally at a dose ranging from 23 mg/kg (0.4 mmol/kg) to 186 mg/kg (3.2 mmol/kg) over a period of six weeks.

Materials and methods

Animals

Forty-two 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 10 weeks prior to the initiation of the experiment, because sexually mature male rats were needed. At 15 weeks of age, a mean body weight of 455 g (383-497 g), the experiment was begun. The animals were housed four of 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 °C, and the air humidity was 50-65%. The rats were provided with CE-2 (Clea Japan, Inc., Tokyo, Japan) and tap water *ad libitum*.

Chemicals

Propylene oxide, a minimum purity of 95%, was purchased from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan).

Treatment

One-fifth of the oral LD_{50} of PO (930 mg/kg)[12] was used as the maximum single dose for intraperitoneal injections in this study, because the intraperitoneal LD_{50} of PO had not been reported. We had confirmed during a preliminary experiment that intraperitoneal injections of 186 mg/kg of PO for five consecutive days were not lethal.

All the rats were weighed and randomly distributed into four treatment groups (23 mg/kg (0.4 mmol/kg), 47 mg/kg (0.8 mmol/kg), 93 mg/kg (1.6 mmol/kg), and 186 mg/kg (3.2 mmol/kg)) plus one control group. Eight or nine animals were assigned to each group. In the case of the treatment groups, PO was dissolved in saline and administered by intraperitoneal injections (5.6 ml of the solution/kg). In the case of the control group, rats received in the same manner, an identical volume of saline only.

At first, we had planned to administer PO to rats in all treatment groups three days a week for six weeks. But after the third injection during the second week, two of the eight rats in the 186 mg/kg group died. Therefore, rats in this group received PO only once a week after the third week, while rats in the other three treatment groups were given PO three days a week for six weeks, according to our initial plan.

Evaluation of testicular toxicity

The rats were sacrificed using an overdose of ether within 24 hours after the final injection of PO or saline 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 Gill's hematoxylin for light microscopical examinations.

The right epididymis was used to investigate sperm morphology[8]. 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 Gill's hematoxylin. From each smear, 1000 sperms were examined and the numbers of sperms with an immature head, sperm with a teratic head, and sperm without a tail were counted. Sperm with an immature head and those with a teratic head were classified according to the method of Mori et al[8].

The left testis and epididymis were used for the sperm count[8].

The testis was dpcapsulated 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 the 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 four times by two experienced researchers, and the mean

values which they obtained were used for our evaluations.

For the measurement of serum testosterone concentration, five rats were randomly selected from the control group and from two of the treatment groups (93 mg/kg and 186 mg/kg), and the serum testosterone concentration of these 15 rats was measured by RIA using a DPC total testosterone kit (Nippon DPC Corporation, Tokyo, Japan).

Statistical analysis

The Two-sample t-test was used for the analysis of organ weight and sperm count. The Mann-Whitney test was used both for the analysis of the rate of sperm with morphological abnormalities and for the analysis of serum testosterone concentration. Test results were interpreted as significant below a level of 0.05.

Results

Body and organ weights

After the third injection during the fourth week, one more rat died in the 186 mg/kg group. Therefore the remaining 39 rats were included for evaluation of the testicular toxicity of PO. In the three rats which died during the treatment period, two which died during the second week had apparent testicular and epididymal atrophy.

The difference in body weight between each treatment group and the control group was not significant at the end of the treatment period (Table 1). Table 1 also shows the testicular and epididymal weights of the rats. Organ weight refers to the summation of the weights of both sides per 100 g of body weight. No difference was found in weight between the right and left testis, or between the right and left epididymis in any of the groups (data not shown). The testicular weight in the 186 mg/kg group decreased compared with the control group, although the difference was not significant. The epididymal weight in the 186 mg/kg group decreased significantly, and a negative trend could be seen between the dose of PO and epididymal weight.

Sperm counts in the testis and the epididymis

Figure 1 compares the sperm counts in the testis and in the two portions (head and body plus tail) of the epididymis. There was no difference in sperm count either in the testis or in the head of the epididymis between each treatment group and the control group. The sperm count in the body plus tail of the epididymis decreased significantly in the 186 mg/kg group, although an apparent negative trend could not be seen between the dose of PO and sperm count.

The rate of sperm with morphological abnormalities

Figure 2 describes 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 186 mg/kg group, the rates of

Table 1 Effects of propylene oxide on body, testicular, and epididymal weights.

The dose of PO (mg/kg body weight)	Body weight (g) (mean (SD))	Testicular weight (g) (mean (SD))	Epididymal weight (g) (mean (SD))
0(n=8)	518.75(28.74)	0.720(0.054)	0.240(0.035)
23(n=9)	506.22(13.89)	0.734(0.054)	0.232(0.015)
47(n=9)	512.11(22.70)	0.711(0.075)	0.230(0.011)
93(n=8)	516.13(18.66)	0.738(0.080)	0.223(0.016)
186(n=5)	503.20(65.03)	0.682(0.054)	0.201(0.015)*

The organ weight refers to the summation of the weights of both sides, per 100g of body weight.
* Significantly different from control ($p<0.05$).

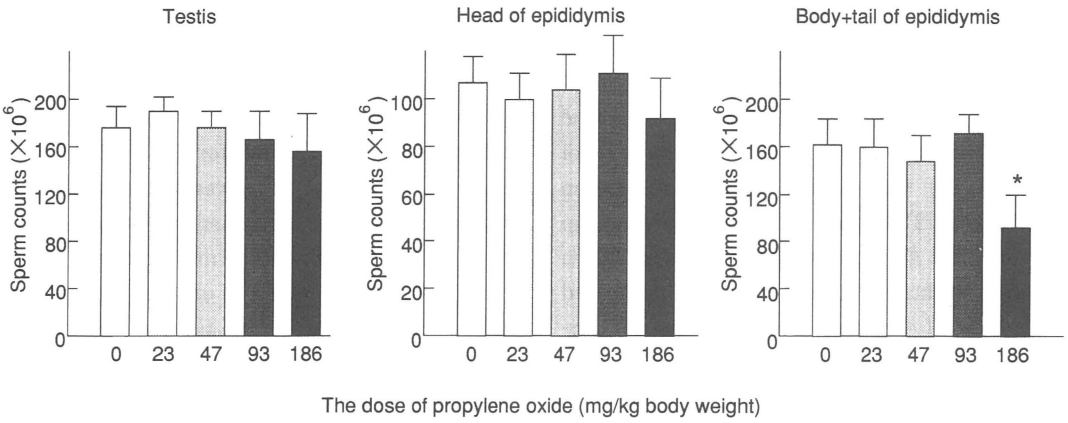

Fig. 1 Effects of propylene oxide on sperm counts in the testis, in the head of the epididymis, and in the body plus tail of the epididymis. All results are described as a mean±SD. Significantly different from control; *p<0.0005.

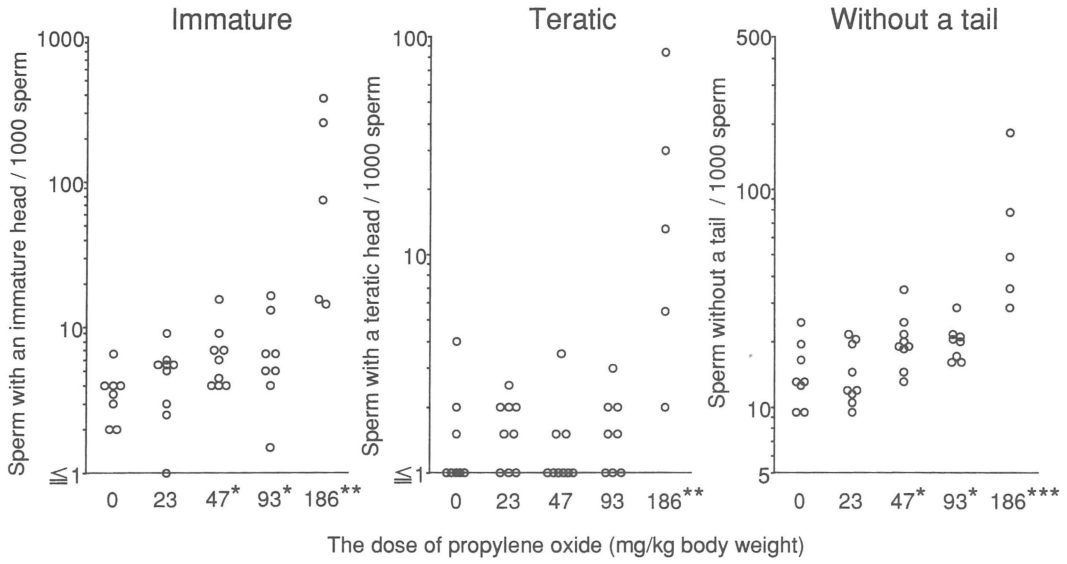

Fig. 2 Effects of propylene oxide on the rate of sperm with morphological abnormalities. Significantly different from control as judged by Mann-Whitney test; *p<0.05, **p<0.01, ***p<0.005.

both sperm with an immature head and sperm with a teratic head increased significantly ($p<0.01$). Moreover, the rate of sperm with an immature head increased slightly, although still significantly, in the 47 mg/kg and in the 93 mg/kg groups, as well ($p<0.05$), and a positive trend could be seen between the dose of PO and the rate of sperm with an immature head. The increase in the rate of sperm without a tail was significant in the 47 mg/kg, 93 mg/kg, and 186 mg/kg groups ($p<0.05$, $p<0.05$, and $p<0.005$, respectively).

Light microscopical findings of the testis

Pathological changes were rarely observed in the seminiferous tubules in any of the treatment groups, with the histological appearance in all treatment groups being indistinguishable from that in the control group. In addition, no appar-

ent pathological changes could be found in Leydig cells or in the interstitium of the testes.

Serum testosterone concentration

The logarithmic means of serum testosterone concentration were 161.4 ng/dl in the control group (n=5), 92.7 ng/dl in the 93 mg/kg group (n=5), and 121.3 ng/dl in the 186 mg/kg group (n=5). The serum testosterone concentration of each treatment group was low compared with that of the control group, however, the difference was not significant.

Discussion

We investigated the dose-dependent testicular toxicity of PO in rats, when injected intraperitoneally. The main findings of this study were: (1) The epididymal weight and sperm count in the body plus tail of the epididymis decreased significantly, while the rate of sperm with morphological abnormalities increased significantly in the 186 mg/kg group; (2) The number of sperm with a teratic head increased only in the 186 mg/kg group. However, the number of sperm with an immature head increased even in the 47 mg/kg group and a dose-dependent effect could be seen; (3) No difference was found in testicular weight or sperm count in the testis between each treatment group and the control group. There were no apparent pathological findings in the seminiferous tubules, Leydig cells, or the interstitium of the testis in the rats which received PO; (4) The serum testosterone concentrations of the rats in the 93 mg/kg and the 186 mg/kg groups were not significantly different from that of the control group.

We at first planned to administer PO three days a week, for six weeks, to rats in all treatment groups. However, the administration frequency had to be reduced to once a week after the third week in the 186 mg/kg group because two of these rats died during the second week. As a result, the administration schedule of this group ceased to be identical with that of the other treatment groups. For this reason, a statistical analysis could not be performed for our evaluation of the dose-dependent effect of PO. In addition, three of eight rats in the same group died during the treatment period and these were not included for the evaluation of testicular toxicity. However, two of these rats, which died during the second week, had apparent testicular and epididymal atrophy. Since even without the inclusion of these two rats the dose-dependent testicular toxicity of PO was confirmed by the results of this study, the effect of their exclusion can only have been to underestimate this toxicity.

In this study, the rate of teratic sperm increased in the 186 mg/kg group. PO is a highly reactive alkylating agent and is known to cause DNA damage[2)3)11)]. Moreover, PO is a low molecular substance (molecular weight: 58.08) which is thought to pass through the blood-testis barrier. It is probable that PO dose pass through the blood-testis barrier to directly affect germ cells in the seminiferous tubules. The number of sperm with an immature head increased even following treatment with 47 mg/kg of PO, a dose lower than that which caused the increase in the number of teratic sperm. An increase in the number of immature sperm is thought to be a result of early spermiation by Sertoli cells, and this type of damage is presumably caused by alterations in the function of Sertoli cells[8)9)]. Therefore, the testicular toxicity of PO is thought to evolve via two different mechanisms, a direct mutagenic effect on germ cells and alterations in the function of Sertoli cells with the latter effect seeming to occur at lower dose. There were no apparent histopathological changes in Leydig cells and no significant decrease in serum testosterone concentration in the treatment groups. Hence, the effect on Leydig cells did not seem to contribute to the testicular toxicity of PO. These results were quite similar to those of our previous studies concerning the testicular toxicity of ethylene oxide[5)8)9)10)]. Probably these two epoxides induce testicular damage via similar mech-

anisms.

The testicular weight and the testicular sperm count did not reduced significantly and there were few pathological findings in the testis in the 186 mg/kg group. However, testicular atrophy was observed in the rats which died during the treatment period after having received 186 mg/kg of PO three days a week for two weeks, and the increase in the rate of immature sperm and that of teratic sperm can not be explained without some effect on the testis. In the 186 mg/kg group, the administration frequency was reduced from three days a week to once a week after the third week. Therefore, recovery from testicular damage may have occurred in the 186 mg/kg group after the third week of treatment. Mori et al. reported the reversibility of ethylene oxide-induced testicular toxicity[10]. However, reversibility of PO -induced testicular toxicity could not be confirmed by the results of this study.

In this study, the rate of sperm without a tail increased following administration with PO at dosage of 47 mg/kg or more. Feuston et al. reported an increase in the number of sperm without a tail following administration with 2-methoxyethanol[4]. However, as far as we know, there have been few studies evaluating the rate of sperm without a tail using sperm taken directly from the epididymis. The epididymis was squeezed to collect sperm for the evaluation of sperm morphology. It is possible that some sperm which originally had their tails may have lost them during the collection procedure. However, there was no difference in the sperm collection procedure between the treatment groups and the control group, and so the increase in the rate of sperm without a tail can not be explained this way. We therefore decided to consider the increase in the rate of sperm without a tail as an index of testicular toxicity. This increase probably reflects an increase in the fragility of the sperm neck.

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(和文抄録)

プロピレンオキシド腹腔内反復投与による ラット雄性生殖機能への影響

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プロピレンオキシド（PO）を雄ラットの腹腔内に反復投与し，その生殖機能に与える影響について調べた．PO投与群は4群に分け，投与量はそれぞれ体重1kgあたり23 mg, 47 mg, 93 mg, 186 mgとし，23 mg～93 mgの3群では週3日・6週間の投与を行ったが，186 mg群では週3回・2週間の投与の後，週1回・4週間の投与を行った．186 mg群では精巣上体の重量および精巣上体体尾部の精子数が有意に減少し，また形態異常を示す精子の割合が有意に増加していた．異常精子のうち未成熟な精子の割合は47 mg以上の投与群で有意に増加しており，POの投与量と未成熟精子の増加との間には量反応関係が見られた．しかし血清テストステロン濃度では対照群と投与群との間には差は見られず，また精巣のLeydig細胞にも病理組織学的な変化は認められなかった．この論文は，POの精巣毒性の量反応関係について調べた初めての報告である．