[Environmental Health and Preventive Medicine 7, 15–18, April 2002]

Original Article

Testicular Toxicity Evaluation of Two Antimony Compounds, Antimony Trioxide and Antimony Potassium Tartrate, in Rats and Mice

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Abstract

Objectives: Testicular toxicities of antimony compounds were evaluated in rats and mice. The slightly water-soluble antimony compound antimony trioxide (ATO) and the highly water-soluble antimony compound antimony potassium tartrate (APT) were examined.

Methods: Daily doses of the compounds were 27.4, 12.0 and 1,200 mg/kg body weight in the APT group, low-ATO group and high-ATO group, respectively. The corresponding daily doses of antimony were 10, 10 and 1,000 mg/kg body weight, in the APT group, low-ATO group and high-ATO group, respectively. Both compounds were administered by gavage: rats, 3 days per week for 4 weeks; mice, 5 days per week for 4 weeks.

Results: Neither compound reduced the weights of reproductive organs or accessory sex organs nor affected sperm parameters. Few marked histopathologic changes were found in the testes of the treated animals. Even at 1,200 mg/kg body weight, which is greater than the LD_{50} of APT, ATO produced no effects.

Conclusions: In this study, it was found that ATO and APT are not toxic to testes in rodents.

Key words: testicular toxicity, antimony compounds, water solubility, rats, mice

Introduction

Antimony compounds are widely used as flame retardants; e.g., in textiles, paper and semiconductor devices[1]. Human daily intake of antimony is estimated to be as high as 1 to 2 μg/kg body weight[1,2], and food and drinking water are the main sources of exposure for the general population[2]. For drinking water, the WHO recommends 0.005 mg/l as the provisional guideline value for antimony, and the USEPA has set 0.006 mg/l as the maximum contaminant level for this metal. The most commercially prominent antimony compound is antimony trioxide (ATO). However, both the WHO-recommended provisional guideline value and USEPA maximum contaminant level for antimony are calculated based on the LOAEL of antimony potassium tartrate (APT) for rats determined in a study by Schroeder et al.[3]. In that study, APT decreased longevity and altered blood levels of glucose and cholesterol. ATO and APT have quite different water solubilities. ATO is slightly soluble in water, whereas APT is highly soluble in water.

Some antimony compounds, including ATO and APT, were reported as genotoxic in vivo[4–7]. During spermatogenesis, the process of sperm production in the testis, active DNA synthesis occurs. Thus, spermatogenesis is vulnerable to genotoxic effects[8]. Gene mutation in germ cells can cause them to die, resulting in transient or permanent sterility, and sometimes causes gene mutation in progeny[9]. As far as we know, there have been few studies on the effects of antimony compounds on the male reproductive system. The genotoxic potency of antimony compounds suggests that they may harm male reproduction. Gurnani et al. reported that 21-day ATO administration induced chromosomal aberrations in bone marrow cells but did not increase sperm head abnormalities in the epididymis of mice[5,6]. However, 21 days is too short an administration period to evaluate toxic effects on spermatogenesis in mice, because it takes approximately 39 days to complete spermatogenesis in mice[10,11]. Thus, the toxic effects of ATO on spermatogonia and early spermatocytes may have been overlooked in those studies. Therefore, in the present study, we evaluated the testicular toxicities of antimony compounds in 2 animal species, rats and mice, after a 4-week administration period. Four weeks are shorter than the period needed to complete spermatogenesis in rats (approximately 8 weeks) and mice (approximately 39 days)[10,11]. However, the International Conference on Harmonisation Tripartite Guidelines on Detection of Toxicity to Reproduction for Medical Products suggest that a 4-week treatment period is appropriate for detection of drug effects on male fertility in rats, provided that adequate histology and organ weight findings are available from repeat-dose studies[12,13]; as described below, we obtained these findings in this study. Therefore, we used 4 weeks as the administration period in this study. To examine the effect of water solubility on the toxicities of antimony compounds, we used

Received Aug. 23 2001/Accepted Oct. 22 2001
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the slightly water-soluble antimony compound ATO and the highly water-soluble antimony compound APT.

Materials and Methods

We purchased ATO (purity greater than 99.999%) and APT (purity greater than 99.5%) from Katayama Chemicals Industries Co. Ltd., Osaka, Japan. We purchased 40 male mice (Crj: CD-1) at 7 weeks of age and 32 male rats (Crj: Wistar) at 6 weeks of age from Kyudo Co., Ltd., Tosu, Japan. After a housing period, animals were randomly divided into 4 groups at 8 weeks of age, the APT group, low-ATO group, high-ATO group and control group, with each group comprising 10 mice and 8 rats. In our preliminary study, mice showed high tolerance for 10-day administration of APT at 274 mg/kg body weight (data not shown). Due to the longer administration period, the daily dose of APT in the present study was 27.4 mg/kg body weight, which is equivalent to an antimony dose of 10 mg/kg body weight. The daily dose of ATO in the low-ATO group was 12.0 mg/kg body weight, which is also equivalent to a dose of antimony of 10 mg/kg body weight. Mice were reported to well tolerate 1,000 mg/kg ATO administration by gavage for 21 days[5,6]. Therefore, the daily dose of ATO in the high-ATO group was 1,200 mg/kg body weight, which is equivalent to a dose of antimony of 1,000 mg/kg body weight. The antimony compounds were suspended or dissolved in distilled water, and were administered by gavage: mice, 5 days per week for 4 weeks; rats, 3 days per week for 4 weeks. Distilled water was administered to the control group in the same manner. The animals were kept in an air-conditioned conventional room (mice) or SPF room (rats). In the conventional room, the light cycle was 12 h light/12 h dark, the temperature range was 24 to 26°C, and the range of air humidity was 40 to 80%. In the SPF room, the light cycle was 12 h light/12 h dark, the temperature range was 22 to 25°C, and the range of air humidity was 50 to 60%. This experiment was reviewed by the Committee on the Ethics of Animal Experiments in the Faculty of Medicine, Kyushu University, and was carried out under the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University, and the Law (No. 105) and Notification (No. 6) of the Government of Japan.

One day after the final administration, the animals were killed by inhalation of carbon dioxide. The testes, epididymides, ventral prostate and seminal vesicle were removed and weighed. The seminal vesicle was weighed without fluid. In addition to the weights of these organs, we evaluated the effects on the male reproductive system using the number, motility and morphology of sperm in the cauda epididymidis and histopathologic changes in the testis. Sperm analysis and the histopathologic examination were performed as described elsewhere[14]. We did not include sperm motility findings of mice in the present report, because the interval between the removal of the epididymis and sperm motility analysis was too long and the findings were therefore thought to be unreliable. In the histopathologic examination of the testis, all round or ovoid cross-sections of the seminiferous tubule (173–449 cross-sections in rats and 55–165 cross-sections in mice) in one transverse section were examined. The following histopathologic changes were examined: disorganization and exfoliation of the seminiferous epithelium, degeneration of germ cells, vacuolization of the epithelium, sperm retention in the epithelium, and delayed spermiation. Statistical differences were analyzed using Fisher's least significant difference procedure after one-way analysis of variance. Differences were considered significant when the p value was below 0.05.

Results

Weights of the testis, epididymis, ventral prostate and seminal vesicle

Two mice and one rat in the high-ATO group and one control mouse died due to accidents at administration. No other animals died during the administration period. Table 1 shows body weight and the weights of the testis, epididymis, ventral prostate and seminal vesicle at the end of the 4-week administration period. We used an absolute organ weight rather than a relative organ weight to evaluate the testis and epididymis weights because these organ weights were independent of body weight[18–20]. The 2 antimony compounds did not affect body weight gain of mice or rats. Seminal vesicle weight was slightly decreased in mice in both ATO groups, but these decreases were not significant. There was no decrease in seminal vesicle weight in rats of either ATO group. In both species, the weights of the other organs in the antimony-treated groups were comparable to the control values.

Table 1 Body weight, reproductive organ weights and accessory sex organ weights of rats and mice at the termination of four-week administration of antimony potassium tartrate (APT) and antimony trioxide (ATO)

	Control	APT[a]	low ATO[a]	high ATO[a]
Rats	n=8	n=8	n=8	n=7
Body weight (g)	402.2±18.7	388.3±25.5	395.6±32.6	399.2±21.9
Testis weight (g)	1.641±0.082	1.703±0.077	1.744±0.161	1.625±0.140
Epididymis weight (g)	0.504±0.042	0.541±0.019	0.540±0.042	0.537±0.042
Ventral prostate weight (g/100 g BW[b])	0.133±0.018	0.136±0.020	0.124±0.026	0.133±0.008
Seminal vesicle weight (g/100 g BW[b])	0.139±0.019	0.140±0.013	0.142±0.016	0.141±0.017
Mice	n=9	n=10	n=10	n=8
Body weight (g)	35.0±0.8	33.7±3.0	34.2±2.9	33.4±2.2
Testis weight (g)	0.249±0.032	0.245±0.034	0.259±0.039	0.238±0.027
Epididymis weight (g)	0.104±0.012	0.099±0.008	0.100±0.009	0.096±0.011
Ventral prostate weight (g/100 g BW[b])	0.048±0.016	0.078±0.033	0.069±0.033	0.086±0.075
Seminal vesicle weight (g/100 g BW[b])	0.879±0.233	0.880±0.137	0.829±0.167	0.662±0.230

[a] Daily administration dose was 27.4 mg, 12.0 mg and 1,200 mg/kg body weight in the APT, low-ATO and the high-ATO group, respectively. Corresponding daily doses of antimony were 10, 10 and 1,000 mg/kg body weight, in the APT group, low-ATO group and high-ATO group, respectively. The antimony compounds were administered by gavage three days per week for four weeks in rats and five days per week for four weeks in mice.

[b] BW means body weight.

Table 2 Sperm count, sperm motility and sperm morphology of rats and mice at the termination of four-week administration of antimony potassium tartrate (APT) and antimony trioxide (ATO)

	Control	APT[a]	low ATO[a]	high ATO[a]
Rats	n=8	n=8	n=8	n=7
Sperm count (×10^6/cauda epididymidis)	133.1±21.7	130.6±16.6	142.2±35.1	140.9±18.7
% motile sperm	83.3±6.6	77.9±9.6	72.4±11.0	77.2±10.6
% progressively motile sperm	60.3±19.6	48.8±10.7	44.5±17.7	52.2±12.4
% sperm head abnormality	0.9±0.3	0.8±0.6	0.7±0.5	0.8±0.3
% sperm tail abnormality	0.5±0.4	0.4±0.3	0.3±0.2	0.3±0.2
% sperm without tail	2.0±0.7	2.0±0.6	1.8±0.8	2.4±1.2
Mice	n=9	n=10	n=10	n=8
Sperm count (×10^6/cauda epididymidis)	31.1±8.6	28.7±8.2	30.2±5.3	28.1±7.4
% sperm head abnormality	2.9±2.4	1.4±1.0	1.9±1.9	2.8±1.9
% sperm tail abnormality	0	0.1±0.1	0.0±0.1	0.1±0.3
% sperm without tail	2.9±2.4	2.7±1.9	2.9±3.1	4.3±5.3

[a] Daily administration dose was 27.4 mg, 12.0 mg and 1,200 mg/kg body weight in the APT, low-ATO and the high-ATO group, respectively. Corresponding daily doses of antimony were 10, 10 and 1,000 mg/kg body weight, in the APT group, low-ATO group and high-ATO group, respectively. The antimony compounds were administered by gavage three days per week for four weeks in rats and five days per week for four weeks in mice.

Sperm count, sperm motility and sperm morphology

Table 2 shows sperm parameters at the end of the 4-week administration period. Neither of the 2 antimony compounds affected the count, motility or morphology of caudal sperm in rats or mice.

Histopathologic changes of the testis

Delayed spermiation was found in one of the 8 rats in the low-ATO group and one of the 7 rats in the high-ATO group. However, the frequencies of delayed spermiation in these 2 rats were less than one percent. One of the 10 mice in the low-ATO group showed exfoliation of the seminiferous epithelium; the frequency in this mouse was greater than 50% (an obvious increase). No mice in the high-ATO group showed an obvious increase in frequency of exfoliation. APT did not cause any histopathologic changes in the testes of rats or mice.

Discussion

In this study, we evaluated the testicular toxicities of ATO and APT in rats and mice after a 4-week administration period. Gurnani et al. reported that ATO did not increase sperm head abnormalities in mice[5,6]. Similarly, neither ATO nor APT increased sperm head abnormalities in rats or mice in the present study. We observed no apparent effects of the 2 antimony compounds on the other sperm parameters or the weights of reproductive organs. We performed detailed histopathologic examination of the testis in this study, because toxic effects on spermatogonia and spermatocytes could be overlooked in a 4-week administration study without this examination[12,13]. One mouse in the low-ATO group showed an apparent increase in the frequency of epithelial exfoliation in seminiferous tubules. However, we did not observe an increased frequency of epithelial exfoliation in mice in the high-ATO group or rats in either of the 2 ATO groups. Therefore, it is suggested that this histopathologic change in the testis is unrelated to ATO administration. In addition, degenerative changes in spermatogonia and spermatocytes were not increased in the animals treated with ATO or APT. Therefore, it is suggested that neither ATO nor APT is toxic to the testes of rats or mice. Mangal and Sohal analysed 10 trace elements (Fe, Zn, Co, Cr, Se, Rb, Sb, Ag, Cs and Sc) in the liver, kidney and testis of rats fed standard diet by neutron activation analysis[15]. Antimony was detected in the liver and kidney, although antimony was not detected in the testes[15]. This suggests that the tissue distribution of ATO and APT in rodents does not involve the testes to a great extent. It is possible that, in the present study, the concentrations of these antimony compounds in the testes did not become sufficiently high for their genotoxicities to result in damage to germ cells.

In this study, even repetitive administration of ATO at a dose of 1,200 mg/kg body weight for a period of 4 weeks was not toxic to testes. Oral LD_{50} of APT in rats and mice is reportedly 115 and 600 mg/kg body weight, respectively[16]. This means that even repeated administration of ATO at a dose exceeding the LD_{50} of APT is not toxic to testes. In this study, we found that antimony concentrations in organs of ATO-treated mice were one-half to one-seventh of those of APT-treated mice[17]. Unlike APT, ATO is only slightly water-soluble and was expected to be far less bioavailable than APT. The difference in water solubility between ATO and APT probably explains the difference in organ concentration, and this solubility difference is probably also responsible for the difference in the toxic effects of these 2 compounds to some degree. It is suggested that distinctions should be made between the toxicities of antimony compounds with different water solubilities when performing hazard assessment of antimony.

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