ATROPINE-INDUCED INHIBITION OF SPERM AND SEMEN TRANSPORT IMPAIRS FERTILITY IN MALE RATS

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ABSTRACT — Previous studies revealed that atropine reduced male fertility in rats without any effects on mating performance, sperm production and motility, and testicular morphology. The present study was conducted to investigate whether the impairment of male fertility induced by atropine was related to the inhibition of sperm and semen transports from the vas deferens and seminal vesicle to the urethra during the process of emission. Male rats were treated with atropine at 125 mg/kg/day for 10-17 days prior to mating with untreated females. After confirmation of mating, male rats were euthanized and sperm number in the vas deferens and weights of the seminal vesicle and copulatory plug were determined as indicators of inhibition of sperm and semen transports, respectively. Reproductive status of mated females was determined on gestation days 15-17. A low pregnancy rate associated with a decreased number of implants was observed in females that mated with the atropine-treated males. The average number of sperm in the vas deferens was increased in the atropine-treated males. The average seminal vesicle weight in the atropine-treated males was greater than that of controls. The copulatory plug weights were decreased in the atropine-treated males. These results suggest that inhibitions of sperm and semen transports from the vas deferens and seminal vesicle to the urethra during the process of emission result in reduced male fertility in rats.

KEY WORDS: Atropine, Fertility, Vas deferens, Seminal vesicle, Sperm transport, Rat

INTRODUCTION

We previously demonstrated an impairment of male fertility induced by atropine, a muscarinic receptor antagonist, in rats (Ban et al., 2002). In the previous study, atropine sulfate was orally administered to male rats at 62.5 and 125 mg/kg/day for 1 week. Males were cohabited with untreated females and bred females were cesarean-sectioned on gestation days 15-17. A low pregnancy rate (69% compared to 100% in controls) associated with a decreased implant number was observed at 125 mg/kg/day. The decreased implant number was also observed at 62.5 mg/kg/day. Because of no effects on mating performance, sperm production and motility and testicular weight and histopathological findings, the mechanism responsible for the impairment of male fertility has not been clarified.

It has been reported that muscarinic receptor antagonists, such as atropine and pirenzepine, inhibit the agonist-evoked contraction of the vas deferens in rats (Doggrell, 1986; Miranda et al., 1995). Atropine also inhibits the contractility of the epididymis produced by acetylcholine in rats (Hib, 1976). The contraction evoked by electrical field stimulation in the rat isolated seminal vesicle was blocked by atropine (Fedan et al., 1977; Gokhale et al., 1996). The contractile inhibition of the vas deferens causes infertility due to reduced sperm transport during the emission phase (Mulryan et al., 2000). When the seminal vesicle was surgically removed, male rats failed to impregnate females due to inhibition of copulatory plug formation (Carballada and Esponda, 1992; Cukierski et al., 1991).

In our previous atropine study, the copulatory plug weights in the atropine groups were decreased in a dose-dependent manner suggesting that the inhibition
of contraction of the seminal vesicle during the process of emission was involved in the reduced male fertility induced by atropine. Therefore, we hypothesized that the effects of atropine on male fertility result from reduced sperm number in ejaculated semen and a reduced semen volume ejaculated due to the contractile inhibition of the vas deferens and seminal vesicle during the emission phase. In order to investigate the hypothesis, we determined (1) the number of sperm in the vas deferens remaining after mating to evaluate the sperm number ejaculated, and (2) seminal vesicle and copulatory plug weights after mating to evaluate the semen volume ejaculated.

MATERIALS AND METHODS

Animals

Male and female Crj:CD(SD)IGS rats were purchased from Charles River Japan Inc., and were housed in an animal room where the temperature (22 ± 2°C) and the relative humidity (55 ± 10%) were controlled. Room lights were set on a 14-hr light and 10-hr dark cycle (lights off 1:00 a.m., lights on 11:00 a.m.). They were fed PMI Certified Rodent Chow #5002 and had free access to tap water throughout the study. Males were fed 22 g/day of the diet except for the cohabitation period when they were fed ad libitum. Females were fed ad libitum throughout the study. Males and females were 10 and 12 weeks of age, respectively, at study initiation.

Experimental designs and treatments

Twenty male rats were orally administered atropine sulfate at 125 mg/kg/day. Atropine (Sigma Chemical Co.; purity, > 97%) was dissolved in distilled water and a factor of 1.17 was used to calculate doses of atropine as the base compound. The dosing volume for all males was 5 mL/kg. Thirteen control males received distilled water. Eight of the control males were assigned to the cohabitation study and 5 other males were used to collect data for vas deferens sperm number and copulatory plug weights after mating to evaluate the semen volume ejaculated.

After 10 daily doses, males were cohabited with untreated females. The cohabitation period was limited to 5 days. Mating was determined by the presence of copulatory plugs or sperm in saline lavage. Dosing was continued until the confirmation of mating. The copulatory plugs on the pan were collected and weighed after drying at 40°C for at least 1 week. Mated males were euthanized by CO₂ just after confirmation of mating (10 to 17 doses in total), and the vas deferens and seminal vesicle were excised and weighed. The vas deferens was frozen at −30°C until sperm head counts. The seminal vesicles were fixed in Bouin’s solution, dehydrated, embedded in paraffin, sectioned at 3 μm thickness, and stained with hematoxylin and eosin for histopathological examinations.

For sperm head counting in the vas deferens, the vas deferens was thawed, minced and homogenized for 4 min in 15 mL of physiological saline containing 1.0% Triton-X 100 (Sigma Chemical Co., MO, USA). The homogenates were centrifuged at 2000 rpm for 20 min. The supernatant was removed and the precipitate sample was diluted to 2 mL at the final volume. An aliquot was dropped into a chamber of hemocytometer after staining with 40% eosin solution, and homogenization-resistant sperm heads from all control and treated males were counted.

The vas deferens and seminal vesicle were excised from 5 control males, which were not scheduled for the mating, to evaluate sperm number in vas deferens and weight and histological examinations of the seminal vesicle as mentioned above.

Mated females were cesarean-sectioned on gestation days 15-17 and the uterus of each female was examined to determine pregnancy status. The number of corpora lutea was counted. Uterine implants were counted and each was classified as a live fetus, dead fetus and resorption.

Statistical analysis

All statistical analysis was performed with Student’s t-test (Snedecor and Cochran, 1967) except for non-parametric data. Non-parametric data for fecundity index [(number of pregnant females/number of females cohabited) × 100] between the control and atropine groups were analyzed using a Chi-square test. Significant differences were determined at a confidence level of p≤0.05.

RESULTS

Vas deferens sperm head count

Fig. 1 shows the average sperm number per gram vas deferens in the non-mated and mated control males, and mated atropine treated males. When the sperm numbers per gram vas deferens are compared between non-mated and mated control males, the sperm number of the mated control group (146.7 ± 192.7×10⁵, n=8) was significantly (p≤0.05) and 74%
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less than that of non-mated males (555.3 ± 65.5 × 10⁵, n=5). The average number of sperm per gram vas deferens in the atropine group (332.0 ± 141.4 × 10⁵, n=20) was significantly (p≤0.05) and 2.3-fold greater than that of mated controls.

Seminal vesicle weights

Fig. 2 shows the average seminal vesicle weights in non-mated and mated control males and mated atropine-treated males. The average seminal vesicle weight in the mated control group (0.94 ± 0.23 g, n=8) was significantly (p≤0.05) and 49% less than that in non-mated males (1.92 ± 0.18 g, n=5). The average seminal vesicle weight in the mated atropine group (1.13 ± 0.36 g, n=20) was not significantly (p>0.05) but 1.2-fold greater than that of mated controls.

Copulatory plug weights

Fig. 3 shows the average copulatory plug weights in the mated control and atropine groups. The average copulatory plug weight in the atropine group (54 ± 38 mg, n=15) was significant (p≤0.05) and 59% less than that in the control group (133 ± 43 mg, n=7).

Histological examination of seminal vesicle

No treatment-related changes were observed in the seminal vesicles from any male rats in the atropine group.

Laparotomy data

Laparotomy data from female rats assigned to mating trails in the control and atropine groups is summarized in Table 1. A treatment-related and significant (p≤0.05) decrease in fecundity index (45%) was observed in the atropine group when compared to 100% in controls. There were treatment-related but non-significant (p>0.05) changes in percentage of pre-implantation loss (13.1%, compared to 3.0% in controls), number of implants per pregnant female (13.3 ± 4.2, compared to 15.5 ± 1.3 in controls) and number of live fetuses per pregnant female (12.4 ± 4.0, compared to 15.0 ± 1.4 in controls) in the atropine group.

There were no treatment-related effects on the mating index, number of corpora lutea per pregnant female, number of resorptions and dead fetuses per pregnant female and percent of postimplantation loss in the atropine group.

DISCUSSION

In the present study, we investigated the possible mechanisms responsible for atropine-induced impairment of fertility in rats. In our previous study (Ban et al., 2002), atropine sulfate was orally administered to male rats at 62.5 and 125 mg/kg/day for 1 week. Males were cohabited with untreated females and bred females were cesarean-sectioned at mid-gestation period. A low pregnancy rate associated with a decreased implant number was observed at 125 mg/kg/day. The decreased implant number was also observed at 62.5 mg/kg/day. Because of no effects on mating performance, sperm production and motility and testic-
ular weight and histopathological findings in atropine-treated males, the mechanism responsible for the impairment of male fertility has not been clarified. In the present study, we hypothesized that atropine inhibits the contraction of vas deferens and/or seminal vesicle during the emission phase, so we evaluated sperm numbers in the vas deferens, and seminal vesicle and copulatory plug weights in male rats after mating.

There were no effects on mating index (\%, number of mated females/females cohabited) and mating behavior (data not shown) of atropine-treated male rats, compared to controls, in the present study. Bignami (1966), however, reported that atropine prevented male rats from mating due to effects on the central nervous system when atropine up to 5 mg/kg was subcutaneously administered 30 min before mating. The plasma kinetics of atropine sulfate after single administration were reported (Urso et al., 1991), where the average maximum plasma concentration (2123.3 ng/mL) was observed at 0.125 hr after an intravenous injection of 10 mg/kg, and the concentration declined thereafter (81.0 ng/mL at 8 hr). On the other hand, when the compound was administered orally at 50 mg/kg, the average maximum plasma atropine level was observed at 0.5 hr (2.50 ng/mL) after dosing and slightly declined thereafter. In the present study, atropine at 125 mg/kg/day was orally administered about 10 hr before mating, indicating there would have been no or little effects of atropine on mating behavior of male rats.

The increased sperm head count in vas deferens observed in the atropine group (Fig. 1) would suggest the partial inhibition of emission phase by atropine treatment. The inhibitory effects of atropine and pirenzepine, another non-selective muscarinic antagonist, on the contractile responses of the epididymal half of the rat vas deferens to methacholine included a depres-

![Graph showing copulatory plug weights](image)

**Fig. 3.** Effect of atropine on copulatory plug weights; the average values in mated control and atropine groups are shown as white, dotted and black columns, respectively. Values are mean ± S.D.. * indicates significantly different from control at p ≤ 0.05 (Student’s t-test).

### Table 1. Reproductive performance of males following treatment with atropine sulfate and reproductive findings in untreated females.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Atropine (mg/kg/day)</th>
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<tbody>
<tr>
<td></td>
<td>0 (control)</td>
</tr>
<tr>
<td>No. of mated females</td>
<td>8</td>
</tr>
<tr>
<td>No. of pregnant females</td>
<td>8</td>
</tr>
<tr>
<td>Fecundity index (Pregnancy rate) (%)</td>
<td>100</td>
</tr>
<tr>
<td>No. of corpora lutea/litter</td>
<td>15.9 ± 1.5(^b)</td>
</tr>
<tr>
<td>Preimplantation loss/litter (%)</td>
<td>3.0 ± 4.5</td>
</tr>
<tr>
<td>No. of implants</td>
<td>124</td>
</tr>
<tr>
<td>No. of implants/litter</td>
<td>15.5 ± 1.3</td>
</tr>
<tr>
<td>No. of resorptions and dead fetuses/litter</td>
<td>0.5 ± 0.8</td>
</tr>
<tr>
<td>Postimplantation loss/litter (%)</td>
<td>3.2 ± 4.9</td>
</tr>
<tr>
<td>No. of live fetuses/litter</td>
<td>15.0 ± 1.4</td>
</tr>
</tbody>
</table>

\(^a\): (No. of pregnant females/no. of mated females) × 100.

\(^b\): Values are given as mean ± SD.

\(^c\): [(No. of corpora lutea - no. of implants)/no. corpora lutea] × 100.

\(^d\): (No. of resorptions and dead fetuses/no. of implants) × 100.

\(^*\): Statistical difference from the control group, p ≤ 0.05.
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...of the maximal response (Doggrell, 1986). Furthermore, acetylcholine-induced contraction of rat epididymis in vivo was blocked by atropine (Hib, 1976). These reports support pharmacological responses of muscarinic receptors in cauda epididymis and vas deferens, and the results in the present study would suggest atropine-induced inhibitory effects on contractile responses in these organs. It has been reported on that effects of reduction of ejaculated sperm number on fertility (MacLeod, 1973), even 90% reduction of ejaculated sperm number is sufficient for impregnating mated females. Therefore, reductions of both ejaculated sperm number and semen volume were responsible for impairment of male fertility induced by atropine.

The average seminal vesicle weight in the atropine group was greater than that of controls (Fig. 2). Correspondingly, the average copulatory plug weight in the atropine group was less than that of controls (Fig. 3). There were no treatment-related histopathological changes of the seminal vesicle, indicating that atropine itself did not affect the secretion of seminal vesicle fluid. Therefore, the increased seminal vesicle weights and decreased copulatory plug weights in the atropine group were considered to be resulted from inhibition of contraction of the seminal vesicle during the process of emission, which is one of the pharmacological effects of the muscarinic receptor antagonists (Sadraei et al., 1995). It has been reported (Gokhale et al., 1996) that contractile responses of rat isolated seminal vesicles induced by electrical field stimulation involve participation of a noradrenergic as well as a cholinergic mechanism, which also supports the results of the present study. The seminal vesicle and copulatory plug play an important role for sperm transport in rats. Sperm transport through the cervix seems to be highly dependent on the formation and position of the copulatory plug, which must be firmly lodged in the cervical opening to allow the passage of spermatozoa into the uterine horns (Matthews et al., 1977, 1978). Carballada and Esponda (1992) demonstrated that a close relationship exists between seminal vesicle size, quantity of seminal secretion in the ejaculate and sperm transport through the cervix. A decrease in the percentage of spermatozoa in the uterus correlated with a reduction in weight of the plug. Therefore, the impairment of fertility induced by atropine would be related to inhibition of contraction of the seminal vesicles, which in turn results in reduced or defective formation of copulatory plugs.

In the atropine group, 8 out of 20 females showed no or few sperm in the vaginal lavage at the confirmation of mating, and 7 of these 8 females were not pregnant. In addition, the copulatory plugs not only small in size but also abnormal in shape, for example, the proximal end of the plug displayed a cup-like shape, were sporadically observed in the atropine group. These cup-like plugs have been reported, where male rats had 25% of seminal vesicle surgically removed, result in significant reduction of live fetuses per pregnant female mated with these males (Carballada and Esponda, 1992). Thus, complete infertility by atropine may be due to the combination effects by sperm number and semen volume ejaculated rather than effects by either sperm number or semen volume.

In the present study, we did not collect the data including vas deferens sperm head count and seminal vesicle weight for the non-mated males in the atropine group. The comparative data in sperm count and seminal vesicle weight between the non-mated and mated males in the atropine group may be made clear by explanation of our hypothesis that atropine pharmacologically affects the contractile inhibition of vas deferens and seminal vesicle during emission. However, the seminal vesicle inhibition in mated males resulted in significantly decreased copulatory plug weight and slightly increased seminal vesicle weight in this study. The decreased copulatory plug weight was also observed in our previous study (Ban et al., 2002). In the case of vas deferens sperm number, since the sperm production, which was evaluated by cauda epididymal sperm count in our previous study (Ban et al., 2002), was not affected by atropine, the increased remaining sperm number in the atropine group would suggest contractile vas deferens inhibition during emission.

The effects of atropine on capacitation ability or genotoxicity to sperms has not been reported. In the present study, we did not examine whether atropine has genotoxicity or affects capacitation ability. Thus, these factors may be involved in atropine-induced impairment of male fertility.

In conclusion, reduction of male fertility in rats induced by atropine treatment was confirmed in the present study. Atropine-treatment to male rats induced increases in sperm numbers remaining in the vas deferens and seminal fluid in the seminal vesicle after mating. These results suggest that one of the mechanisms for the impairment of male fertility induced by atropine includes the contractile inhibition of the vas deferens and seminal vesicle during the process of emission.
REFERENCES


