EFFECT OF 4-CHLOROMETHYL-2-METHYL-2-PENTYL-1,3-DIOXOLANE (AY-22,352) ON THE FERTILIZING ABILITY OF HAMSTER EPIDIDYMAL SPERMATOZOA

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Summary. Fertile male hamsters were injected subcutaneously with AY-22,352 to determine the minimal antifertility dose, the site of action and the onset and duration of infertility. Fertility tests showed that 37 mg AY-22,352/kg/day induced sterility within 4 days. None of the males became infertile within the first 2 days but marked loss of sperm fertilizing ability had occurred by 24 hr from the second injection; all males were sterile after the fourth dose. Comparable daily treatment produced the same antifertility effect subsequent to bilateral ligation of the corpus epididymidis and fertilization did not occur after artificial insemination with spermatozoa from the cauda epididymidis of such animals. The prompt recovery of fertilizing ability of spermatozoa in males which received four daily doses of AY-22,352 (37 mg/kg) and had ligated ductuli efferentes shows that transport of epididymal spermatozoa and their acquisition of fertilizing ability are not influenced by the drug.

INTRODUCTION

It has been recently shown that 66 mg 1-chloro-2,3-propanediol (α -chlorohydrin)/kg/day injected subcutaneously for 4 days is the minimal dose which inhibits the fertilizing ability of spermatozoa in male hamsters (Lubicz-Nawrocki & Chang, 1974a, b). In a limited study in male rats, Banik, Tanikella & Rakhit (1972) reported that a closely related compound, 4-chloromethyl-2-methyl-2-pentyl-1,3-dioxolane (AY-22,352), was a more effective chemosterilant on a molar basis than α -chlorohydrin. The present study was undertaken to test the antifertility effect of AY-22,352 in hamsters, to elucidate its site of action and to determine the onset and duration of sterility after sequential daily treatment. In addition, tests were carried out to determine whether AY-22,352 influences transit of spermatozoa through the epididymis and their capacity to acquire fertilizing ability. The chemical formulae and structures of α -chlorohydrin and AY-22,352 are presented in Text-fig. 1.

TEXT-FIG. 1. The chemical structure and formula of (a) α -chlorohydrin and (b) AY-22,352.

MATERIALS AND METHODS

Adult male and female hamsters (125 to 145 g) were maintained under controlled conditions of temperature and light. At the beginning of each experiment, AY-22,352 (density 1.010; Ayerst Research Laboratories) was diluted with dimethylsulphoxide (DMSO) to the desired concentration. Groups of five males of known fertility were injected subcutaneously with the appropriate daily dose in 0.1 ml/100 g body weight while control males received the same volume of DMSO.

To determine the minimal antifertility dose of AY-22,352, pilot experiments were performed on male hamsters which received various doses of AY-22,352 daily for 4 days. Finally, two groups of males were treated daily with 32 and 37 mg AY-22,352 and paired with females in oestrus 24 hr after the fourth injection. Mating was confirmed by the presence of spermatozoa in the vagina and females were killed approximately 40 hr after mating. Eggs were flushed from the uterine tubes with 0.2 ml Hanks' solution and examined with a phase-contrast microscope for evidence of fertilization (the presence of a spermatozoon in the vitellus of a cleaved egg). Throughout this study fertilizing ability was expressed in terms of fertilization rate, i.e. the percentage of fertilized eggs recovered from five females.

To test whether AY-22,352 affected spermatozoa in the cauda epididymidis and ductus deferens, the corpus epididymidis was bilaterally ligated in ten hamsters (Lubicz-Nawrocki & Glover, 1973). Five of the animals were injected daily with AY-22,352 whilst the others received DMSO only. Treated and control males were permitted to mate on the 5th day after ligation.

To determine the time of onset of the antifertility effect in relation to the number of daily injections of AY-22,352 needed to induce infertility, males in four groups were injected daily with 37 mg AY-22,352/kg for 1 to 4 days. Males from each group were paired with oestrous females 24 hr after the first or last injection.

In a fourth experiment to test the fertilizing ability of spermatozoa from the cauda epididymidis by artificial insemination, five males received four daily injections of 37 mg AY-22,352/kg and five were injected with DMSO. Animals in both groups were killed 24 hr after the last injection and ten sperm suspensions were prepared, one from each animal, by macerating both caudae in

3 ml sterilized Hanks' solution. After filtration through sterilized gauze, each sperm suspension (0.2 ml) was then injected into both uterine horns of a female under pentobarbitone sodium anaesthesia (60 mg/kg, intraperitoneally). The number of spermatozoa inseminated was estimated from haemocytometer counts.

To investigate the duration of infertility after treatment, five intact males were rendered infertile by four daily injections of AY-22,352 (37 mg/kg); they were mated 24 hr after the last dose and again 4 days later. Control animals were similarly mated after four daily injections of DMSO.

Since fertilizing ability returned within 5 days after the end of treatment with AY-22,352 (see Table 5) and most spermatozoa in the corpus epididymidis are known to be infertile (Horan & Bedford, 1972), the effect of AY-22,352 on the competence of epididymal spermatozoa to acquire fertilizing ability was tested. Bilateral ligation of the ductuli efferentes with sterilized braided silk sutures (size 5/0, Ethicon) was carried out in ten males. The ligatures were placed close to the testis with minimal disturbance to the vasculature. Five males received 37 mg AY-22,352/kg for 4 days and five were given DMSO. Each male was paired as described above. Males of both groups were then killed and the epididymides from each animal were macerated in 1.5 ml saline with fine scissors; 0.05 ml sperm suspension was diluted to 10 ml with 10% formalin and the total sperm content was estimated from haemocytometer counts.

Tests were then carried out in order to ascertain if the AY-22,352 treatment (37 mg/kg/day for 4 days) produced a second period of infertility and treated males were serially mated each week (Jackson, 1966) using five males for 9 weeks after the last injection of the drug.

RESULTS

Table 1 shows that daily subcutaneous injection of 32 mg AY-22,352/kg for 4 days impaired fertilizing ability. None of the males became sterile, but only 42% of eggs were fertilized. At 37 mg/kg, however, each male was sterile after the fourth dose, as were males similarly treated after ligation of the corpus epididymidis (Table 2).

Table 1. Fertility of male hamsters injected with different doses of AY-22,352 for 4 days

Dose (mg/kg/day)	No. of females mated*	No. of eggs recovered†	No. of eggs fertilized	Fertilization rate‡
DMSO (control)	5	54	54	100
32	5	50	21	42
37	5	54	0	0

DMSO = dimethylsulphoxide.

* Each female was paired with a treated male.

† From five females/group killed 40 hr after mating.

‡ Expressed as the % of eggs fertilized.

Table 2. Fertility of male hamsters 4 days after bilateral ligation of the corpus epididymidis and daily treatment with AY-22,352

<i>Treatment (five males /group)</i>	<i>No. of females mated*</i>	<i>No. of eggs recovered*</i>	<i>No. of eggs fertilized</i>	<i>Fertilization rate*</i>
DMSO* (control)	5	60	60	100
AY-22,352 (37 mg/kg)	5	59	0	0

* See Table 1.

Table 3. The onset of sterility in male hamsters treated with AY-22,352

<i>Treatment*</i>	<i>No. of days of treatment</i>	<i>No. of females mated†</i>	<i>No. of eggs recovered†</i>	<i>No. of eggs fertilized</i>	<i>Fertilization rate†</i>	<i>No. of males becoming infertile</i>
DMSO† (control)	4	5	59	59	100	0
AY-22,352	1	5	60	60	100	0
	2	5	56	41	73.2	0
	3	5	58	1	1.7	4
	4	5	59	0	0	5

* Five males/group given 37 mg AY-22,352/kg/day and mated 24 hr after the last injection.

† See Table 1.

Table 4. Fertilizing ability of spermatozoa in the cauda epididymidis of males treated daily with 37 mg AY-22,352/kg for 4 days

<i>Treatment (five males /group)</i>	<i>No. of sperm. × 10⁶/uterine horn (Mean ± S.E.)</i>	<i>No. of females inseminated†</i>	<i>No. of eggs recovered*</i>	<i>No. of eggs fertilized</i>	<i>Fertilization rate*</i>
DMSO (control)	10.9 ± 2.1	5	61	61	100
AY-22,352	14.2 ± 3.1	5	58	0	0

* See Table 1.

† Each female was artificially inseminated with spermatozoa from one treated male.

Spermatozoa from males given one to four daily injections became progressively incapable of fertilizing eggs, the effect being complete 24 hr after the fourth dose (Table 3). Table 4 shows that spermatozoa in the cauda epididymidis were affected by the chemical, whilst Table 5 shows that sterile hamsters regained fertility within 5 days from the last dose. Bilateral ligation of the

Table 5. The resumption of fertilizing ability of spermatozoa from male hamsters treated with AY-22,352

<i>Treatment*</i>	<i>No. of eggs recovered</i>	<i>Fertilization rate†</i>	<i>Days after last injection</i>	<i>No. of eggs recovered</i>	<i>No. of eggs fertilized</i>	<i>Fertilization rate†</i>
DMSO (control)	52	100	5	58	58	100
AY-22,352	56	0	5	54	54	100

* Five males/group given 37 mg AY-22,352/kg/day for 4 days.

† See Table 1.

Table 6. The effect of bilateral ligation of the ductuli efferentes and treatment with AY-22,352 on the fertility of male hamsters

<i>Treatment*</i>	<i>No. of females mated†</i>	<i>No. of eggs recovered</i>	<i>Fertilization rate†</i>	<i>No. of sperm. $\times 10^6$ in both epididymides and ductus deferens (Mean $\pm$ S.E.)‡</i>
Males tested 1 day after last injection				
DMSO† (control)	5	57	100	—
AY-22,352	5	59	0	—
Males tested 5 days after last injection				
DMSO† (control)	5	58	100	13.8 $\pm$ 1.5
AY-22,352	5	51	100	15.4 $\pm$ 6.2

* Five males/group given 37 mg AY-22,352/kg daily for 4 days.

† See Table 1.

‡ Following the second mating test 5 days after the last injection.

ductuli efferentes alone did not impair the fertilizing ability of spermatozoa whereas males also treated with AY-22,352 (four doses of 37 mg/kg) were infertile on Day 5 after the operation, but fertile matings again occurred 5 days from the last dose (Table 6). The mean number of spermatozoa remaining in the epididymis and ductus deferens was similar in drug-treated and control males.

Weekly matings of males that previously were rendered sterile with AY-22,352 showed that fertile matings occurred for at least 9 weeks after the end of treatment.

DISCUSSION

In the rat, AY-22,352 was found to be a more effective chemosterilant than α -chlorohydrin (Banik *et al.*, 1972). The present results compared with the antifertility effects of α -chlorohydrin in the hamster (Lubicz-Nawrocki & Chang, 1974a, b) show that this analogue of α -chlorohydrin is almost twice as effective in inducing sterility. Like α -chlorohydrin, AY-22,352 caused loss of fertilizing ability of hamster spermatozoa in the cauda epididymidis since ligation of the corpus epididymidis did not affect the antifertility effect of the drug, nor were eggs fertilized after artificial insemination of caudal spermatozoa from treated males receiving either compound. The onset and duration of infertility after sequential daily injection of hamsters with AY-22,352 (37 mg/kg) is similar to that following treatment with 66 mg α -chlorohydrin/kg (Lubicz-Nawrocki & Chang, 1974b). Fertile matings also occurred 5 days after the last injection of 37 mg AY-22,352/kg both in intact hamsters and in males with bilaterally ligated ductuli efferentes. Although it is possible that AY-22,352 prevented sperm maturation during the period of administration, the prompt recovery of fertility in these animals was probably due to passage of unaffected spermatozoa from the caput and corpus epididymidis into the caudal region of the duct. However, it is not clear whether α -chlorohydrin and AY-22,352 affect epididymal function so it is possible that, with time, the effects of these drugs wear off and this would account for the recovery of fertility. No evidence of an adverse effect on spermatogenesis was seen because of the normal fertility for 9 weeks from the end of treatment.

Alpha-chlorohydrin is unsuitable as a male chemosterilant because of its toxic side effects but it is feasible that less harmful analogues will be synthesized for the chemical control of male fertility. As yet, there is no information as to whether AY-22,352 produces the same undesirable side effects as α -chlorohydrin (U. K. Banik, personal communication). In terms of antifertility activity, however, this study shows that AY-22,352 inhibits fertilizing ability more effectively on a molar basis than does α -chlorohydrin (Lubicz-Nawrocki & Chang, 1974a).

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