Fertility control in laboratory rats and mice after feeding with the antigestagen RU486

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The intermittent use of an antigestagen could prove to be a very effective way of controlling the fertility of rats and mice in the wild. This concept was tested by giving paraffin wax blocks containing cereal grains and the antigestagen Mifepristone, RU486 (150 mg kg^{-1} block) to male and female laboratory rats and mice in a series of free-choice feeding experiments. There was no significant difference in the consumption of blocks with or without RU486, showing that it was completely palatable to rats and mice, and no aversion developed following refeeding. The average consumption of RU486 by rats was $11 \text{ mg kg}^{-1} \text{ day}^{-1}$; mice consumed $37 \text{ mg kg}^{-1} \text{ day}^{-1}$. All the females showed persistent oestrous vaginal smears throughout the treatment. When male and female rats and mice were given continuous access to treated paraffin blocks for 30 days, no conceptions occurred. At the end of this time, there was a significant increase in ovarian weight in the treated rats and mice, but no difference in testicular weight. Treated blocks were given to rats for 3 days every 21 days for a total of 115 days. Four dead litters were produced following the first antigestagen treatment on day 21, but no more litters were produced and no treated rats were pregnant when autopsied on day 115. Mice were initially treated for 3 days every 21 days, but some animals continued to produce live young on this schedule. The treatment period was therefore reduced to 3 days every 18 days and no more litters were produced and none of the treated females was pregnant at autopsy. The antigestagen RU486 shows considerable promise as a chemosterilant for the control of fertility in female rats and mice. Intermittent administration every 18 days (mice) or 21 days (rats) in free-choice feeding trials completely inhibited reproduction.

Introduction

RU486, one of a group of antigestagens, has been extensively studied in laboratory animals and in women for its abortifacient properties (Kovacs *et al.*, 1984; Neef *et al.*, 1984; Philibert *et al.*, 1985; Beier *et al.*, 1986; Chwalisz and Elger, 1986; Van Look and Bygdeman, 1989; Ulmann *et al.*, 1990). It has also been shown that daily administration at low dosages inhibits ovulation in monkeys and women (Croxatto *et al.*, 1993). We proposed that antigestagens might therefore prove useful as chemosterilants for the control of animal populations (Gao and Short, 1993a). Theoretically, it would only be necessary to administer the compound intermittently, at an interval determined by the duration of gestation of the species in question, to inhibit reproduction. This would make it ideally suited for use in seasonally monoestrous species like foxes, where it would only need to be administered once a year (Gao and Short, 1993a).

Since RU486 is known to be a potent antigestagen in rats and mice, we investigated whether it was palatable to rats and mice, and whether an intermittent dosage regimen could be developed that would completely inhibit reproduction.

Materials and Methods

Animals

Sexually mature male and female Long Evans rats and BALB/c mice were used; the animals were kept in a cycle of 12 h light:12 h dark and given water *ad libitum* and a standard laboratory diet (G2 + ; King and Co., North Melbourne). This balanced diet consisted of wheat meal, soybean meal, linseed meal, rice pollard, meat meal, molasses, vitamins, minerals, salts, calcium and phosphorus.

Steroid

RU486 (Mifepristone) was a gift from A. Ulmann (Roussel-Uclaf, Paris).

Baits and diet

Paraffin wax (Paraplast: melting point 56°C , Monoject Scientific, St Louis, MO) was first melted in an oven at 65°C . The required amount of RU486 was added to the melted paraffin which was stirred on a hot plate for 20 min. A mixture of blended cereals was then added to give a ratio of 26% paraffin:74% mixed cereals (wheat meal 29%, corn meal

28%, oat meal 28%, canary seed 7%, sugar 7% and defatted milk powder 1%). After mixing well, blue cake and confectionery dye (Queen Fine Foods Pty Ltd, Queensland) was added for the purpose of identification (3 µl dye:1 g cereal-paraffin mixture). The paraffin mixture was then poured into plastic dishes, 7 cm square and 1 cm deep and allowed to set, to produce individual paraffin blocks weighing approximately 35 g. The concentration of RU486 in the blocks was 150 mg kg⁻¹ block. Untreated control paraffin blocks were prepared in the same way but without the addition of steroid.

Treatment groups

Palatability of RU486 paraffin blocks. Twelve adult female rats were housed individually and vaginal smears were examined for 5 days to assess the normality of their oestrous cycles. They were then weighed and assigned at random to control or treatment groups of six animals. Twelve adult male rats were also weighed and caged singly, and assigned randomly to control or treatment groups of six animals. In addition to the standard laboratory diet, all animals were given either the untreated or treated paraffin blocks for three periods of 6 days, separated by periods of 2 days with no treatment. This 2 day interval was to assess re-acceptability of the bait. One block was suspended inside each cage by a wire. Food intake of the laboratory diet and paraffin blocks was recorded daily throughout the treatment period by weighing the food remaining in the hoppers and weighing the paraffin blocks. Any broken-off pieces of paraffin block in the bottom of the cage were separated from faeces and weighed.

During the treatment periods, vaginal smears were taken daily to determine whether ingestion of RU486 could inhibit ovulation. On day 23, after the last day of treatment, control males and females and treated males and females were paired together in cages with sawdust bedding. Dates of birth and number of offspring were recorded as an index of post-treatment fertility.

An identical experiment was performed with twelve male and twelve female mice.

Antifertility effects of RU486. Eighteen male and eighteen female rats were weighed and assigned at random to a control group or to treatment groups A and B, each of six pairs of animals. In addition to the standard laboratory diet, animals in the control and treatment group A were given untreated or treated paraffin blocks daily from the day of pairing for 30 days and then were killed. Their testes, epididymides and ovaries were weighed. The females were examined for pregnancy. The animals in treatment group B were first given the treated blocks for 3 days starting 22 days after pairing. Thereafter, they were given the treated blocks daily for 3 days every 21 days. Throughout the course of the experiment consumption of the paraffin blocks was recorded, together with the dates of birth and number of offspring. On day 109, the male rats were killed by CO₂ and their testes and epididymides weighed. On day 115, the females were killed by CO₂, the ovaries weighed, and the number of pregnant females and number of fetuses in a litter were recorded.

A similar trial was carried out in mice, with the exception that from day 82 after the start of treatment, the non-treatment

period was reduced from 21 days to 18 days. Male mice were killed by CO₂ on day 103 and females on day 109.

Analysis of data. Results are shown as means ± SEM throughout. Comparisons between means of control and treatment groups were made using the unpaired *t* test, unless otherwise indicated. 'Not significant' implies *P* > 0.05.

Results

Palatability of RU486 paraffin blocks

The treated paraffin blocks were as palatable as untreated control paraffin blocks for both male and female rats in a free-choice feeding system (Fig. 1). The consumption of control and treated blocks was usually higher on the first day of the experiment than on subsequent days. The average consumption of the treated blocks by male rats was 20.7 g per rat per day, giving an effective consumption of RU486 of 10.8 mg kg⁻¹ body weight day⁻¹; for treated female rats, block consumption was 13.1 g per rat per day, with an RU486 consumption of 11.3 mg kg⁻¹ body weight day⁻¹. Animals ate over three times more of the standard laboratory diet on days when the paraffin blocks were not given than on the days when they were available (Table 1), indicating the high palatability of the paraffin blocks. Indeed, both control and treated paraffin blocks were much more palatable than the standard laboratory diet.

The treated paraffin blocks were also just as palatable to mice as the untreated paraffin blocks (Fig. 2). The average consumption of the treated blocks by male mice was 6.6 ± 0.5 g per mouse per day, and for female mice it was 5.0 ± 0.2 g per mouse per day. Thus the average consumption of RU486 was 37.4 mg kg⁻¹ body weight day⁻¹ for the males and 36.4 mg kg⁻¹ body weight day⁻¹ for the females. As in the experiments with rats, the mice ate significantly more of the standard laboratory diet on the days when paraffin blocks were unavailable than when the blocks were available (Table 1), indicating the high palatability of the control and treated paraffin blocks.

Before the start of treatment, both control and treated females experienced normal oestrous cycles, as judged by their vaginal smears. During the treatment period, the control female rats continued to have normal oestrous cycles, whereas all the treated female rats showed constant oestrous vaginal smears from day 4 of treatment until its end, including the periods of 2 days with no treatment. At the end of the treatment, all the female rats in both groups gave birth to normal young. Their litter sizes were similar: 8.8 ± 1.0 and 9.5 ± 1.6 young per litter in the control and treatment groups, respectively. However, the treated females gave birth 35.2 ± 3.7 days after being caged with males; this was significantly later than the 26.0 ± 0.5 days of the control animals, presumably because it takes some time for ovarian follicular development to return to normal following cessation of treatment.

During the experiment, one female mouse in the control and in the treatment groups died of apparently unrelated causes. At the end of treatment, all the mice gave birth to normal young. The litter sizes, although smaller in the treated group (5.6 ± 1.7

Fig. 1. Daily consumption (mean $\pm$ SEM) of untreated paraffin blocks or paraffin blocks containing 150 mg RU486 kg⁻¹ block by rats in a free-choice feeding trial in the presence of their standard laboratory diet for three periods of 6 days separated by intervals of 2 days with no treatment. (○) control male rats; (●) treated male rats; (□) control female rats; (■) treated female rats.

Table 1. Consumption of standard laboratory diet depending on whether control or RU486-treated paraffin blocks were available (g per animal per day)

Animals	Paraffin blocks available	Paraffin blocks not available	P
Control male rats	3.2 $\pm$ 0.4	18.7 $\pm$ 0.8	< 0.0001
Treated male rats	4.1 $\pm$ 0.3	18.4 $\pm$ 0.5	< 0.0001
Control female rats	2.8 $\pm$ 0.3	12.2 $\pm$ 0.5	< 0.0001
Treated female rats	4.0 $\pm$ 0.4	13.0 $\pm$ 1.4	< 0.0001
Control male mice	0.5 $\pm$ 0.1	3.7 $\pm$ 0.4	< 0.0001
Treated male mice	0.4 $\pm$ 0.1	3.4 $\pm$ 0.4	< 0.0001
Control female mice	0.4 $\pm$ 0.1	3.1 $\pm$ 0.3	< 0.0001
Treated female mice	0.5 $\pm$ 0.1	2.9 $\pm$ 0.4	< 0.0001

young versus 8.0 ± 1.6 young), were not significantly different from those of the controls. However, the treated females gave birth 31.2 ± 2.9 days after being caged with males; this was somewhat later than the 24.2 ± 2.4 days of the controls, although the difference was not statistically significant.

Antifertility effects of RU486

Continuous ingestion of RU486 in paraffin blocks for a month in a free-choice feeding system resulted in total prevention of conception in rats. None of the treated females in treatment group A was pregnant at the end of one month. In the intermittent feeding protocol, when treatment was withheld for the first 21 days following pairing, four out of the six rats in treatment group B gave birth, but in two of the four litters all of the litter were dead. After this, none of the treated females gave birth, whereas all of the control females gave birth to another three litters (Table 2). Autopsy revealed that

five out of the six control female rats were pregnant, whereas none of the treated females in treatment group B was pregnant.

In mice, none of the treated females in treatment group A was pregnant at autopsy at the end of 30 days of continuous exposure to RU486. After intermittent feeding, the controls produced four litters during the treatment period of 102 days (Table 3). One of the treated female mice in group B died during the first labour. Three of the five remaining mice gave birth to live young (Table 3). Three mice also produced a second litter, although somewhat later than the controls (Table 3). It was therefore decided to reduce the treatment interval from every 21 days to every 18 days. After this, none of the treated animals produced any more young, and at autopsy none of them was pregnant, whereas four of the six controls were pregnant.

There was no significant difference in weights of testes or epididymides between control and treated animals after 30 days of continuous treatment. However, the ovaries of the

Fig. 2. Daily consumption (mean $\pm$ SEM) of untreated paraffin blocks or paraffin blocks containing 150 mg RU486 kg⁻¹ block by mice in a free-choice feeding trial in the presence of their standard laboratory diet for three periods of 6 days separated by intervals of 2 days with no treatment. (○) control male mice; (●) treated male mice; (□) control female mice; (■) treated female mice.

treated animals were significantly heavier than those of the controls, owing to the increased amount of luteal tissue. The paired ovarian weight in control rats was 87.7 ± 9.0 mg versus 120 ± 8.3 mg ($P < 0.01$) in the treated rats. The ovaries of control mice weighed 11.2 ± 1.0 mg versus 25.8 ± 4.6 mg ($P < 0.05$) in the treated mice.

Discussion

These experiments demonstrate conclusively that RU486, when incorporated into paraffin wax blocks containing an admixture of cereal grains to give a concentration of 150 mg RU486 kg⁻¹ paraffin block, is completely palatable to rats and mice, and is eaten in preference to their standard laboratory diet. At this concentration, rats consumed a daily dose of about 12 mg RU486 kg⁻¹ body weight, and mice 37 mg kg⁻¹. All the treated female rats and mice developed oestrous vaginal smears and their ovaries were significantly heavier because of an increased amount of luteal tissue; testicular weight was unaffected. Philibert *et al.* (1985) and van der Schoot *et al.* (1987) also recorded a significant increase in rat ovarian weight following chronic RU486 treatment. In the study reported here, treated paraffin blocks provided intermittently, for 3 days every 21 days to rats, and for 3 days every 18 days to mice, prevented the birth of any live young.

Antigestagens like RU486 therefore have a number of significant advantages over androgens, oestrogens or gestagens for rodent fertility control. Methyltestosterone is relatively unpalatable at the concentration required to induce infertility; ethinyl oestradiol causes complete bait rejection; and Org 5933, a potent orally active gestagen, although completely palatable, has to be fed continuously to inhibit ovulation, and fertility returns rapidly following the cessation of treatment (Gao and Short, 1993b). The complete inhibition of rodent reproduction by the intermittent use of an anti-gestagen is likely to make this a much more cost-effective way of controlling rodent reproduction. In the accompanying paper (Gao and Short, 1994), we demonstrated that the intermittent

Table 2. Fertility of rats given paraffin blocks containing 150 mg RU486 kg⁻¹ block for 3 days every 21 days

Group	First litter	Number of rats giving birth and litter size			Fourth litter
		Second litter	Third litter		
Control ($n = 6$)	6	6	6	6	
Litter size	11.0 ± 1.2	9.0 ± 1.7	10.2 ± 1.3	10.7 ± 0.6	
Treated ($n = 6$)	2	0	0	0	
Litter size	9.5	—	—	—	

The first treatment started on day 21 after pairing.

Table 3. Fertility of mice given paraffin blocks containing 150 mg RU486 kg⁻¹ block for 3 days every 21 days or 18 days

Group	First litter	Number of mice giving birth and litter size			Fourth litter
		Second litter	Third litter		
Control ($n = 6$)	6	6	5	3	
Litter size	6.5 ± 0.7	9.5 ± 1.1	8.2 ± 1.2	8.3 ± 0.7	
Treated ($n = 6$)	3	3	0	0	
Litter size	10.3 ± 0.9	7.7 ± 2.0	—	—	

The first treatment started on day 21 after pairing. After birth of the second litter to the treated animals, the treatment interval was reduced to 18 days.

administration of RU486 is much more effective in inhibiting the reproduction of wild mice in large outdoor enclosures than is the continuous administration of methyltestosterone.

One major advantage of the antigestagens in comparison to all other steroidal chemosterilants is that they show a degree of species specificity. For example, RU486 does not show high-affinity binding to the endometrial progesterone receptors of chickens, hamsters, cats, horses, wallabies or Australian native cats (Gao and Short, 1993a and unpublished observations). In chickens and hamsters, this lack of binding has been shown to be due to a single amino acid substitution at position 575 in the receptor molecule; if the cysteine of the chicken or hamster is replaced by glycine, RU486 binding and antigestagenic activity is restored (Benhamou *et al.*, 1992). Thus it might be possible to select an antigestagen that was relatively specific for the target species, thereby reducing the problem of secondary effects following accidental ingestion by non-target species.

The primary site of action of RU486 in controlling rodent fertility, especially following intermittent administration, is presumably the uterus. Competitive binding to the uterine progesterone receptor will result in increased uterine contractility and induction of abortion. The fact that all the treated rats and mice in the study reported here showed oestrous vaginal smears is presumably a consequence of the withdrawal of progestagenic influence on the vaginal epithelium.

Although it has been claimed that chronic RU486 administration does not block ovulation in rats (Philibert *et al.*, 1985; van der Schoot *et al.*, 1989), it has been shown that RU486 blocks the action of progesterone on the granulosa cells of the Graafian follicles of rats, thereby inhibiting the development of 3β -hydroxysteroid dehydrogenase activity. This appears to block preovulatory progesterone production and hence the induction of the preovulatory surge of LH and ovulation (Tanaka *et al.*, 1993). When women were given a continuous low dose of RU486 of $5\text{--}10\text{ mg day}^{-1}$, follicular growth was arrested and ovulation inhibited; after the cessation of treatment, follicular maturation resumed, and there was an LH surge followed by ovulation (Croxatto *et al.*, 1993). In the studies reported here, none of the rats or mice on continuous RU486 treatment became pregnant. However, they all conceived and had normal size litters, soon after the cessation of treatment, although there was a delay in conception of 7–9 days compared with the controls. We could not determine whether ovulation was inhibited, but the volume of luteal tissue increased in rats and mice following chronic administration.

In conclusion, it seems that the intermittent use of antigestagens like RU486 holds much promise as a novel way of regulating rodent fertility.

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