SOME BIOLOGICAL PROPERTIES OF RMI 12,936, A NEW SYNTHETIC ANTIPROGESTATIONAL STEROID

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Summary. A new synthetic steroid, RMI 12,936, was examined for its contraceptive potential in rats and comparisons with ethinyloestradiol were also made. Marked differences in the biological effects of the compounds were found, RMI 12,936 having high antiprogestational but negligible oestrogenic activity. Administration of RMI 12,936 on Day 1 of pregnancy caused acceleration of egg transport, the initial changes being apparent within 12 hr of dosing. Termination of pregnancy was associated with a significant reduction in ovarian weight. On Day 8 of pregnancy, RMI 12,936 resulted in significant ovarian hypertrophy, apparent within 48 hr, possibly due to a luteotrophic stimulus of placental origin. Pregnancy could not be maintained by progesterone implants, indicating that utilization was inhibited. Egg transfer experiments indicated that the primary effects were probably on the maternal reproductive tract. Pregnancy was terminated after administration of RMI 12,936 on Day 19.

The compound also had antifertility activity following intravaginal administration.

INTRODUCTION

Pharmacological means of contraception have not yet achieved the ideal of complete efficacy without side-effects. Continuous administration of low doses of progestins offer the closest approach at present, but the problems of comparatively low efficacy and incidence of endometrial side-effects persist (see Mears, Vessey, Andolsek & Oven, 1969; Board, 1971). Animal studies have indicated that this regimen probably advances the changes characteristic of early pregnancy in the maternal reproductive tract, causing asynchrony between the development of the ovum and that of its environment (Kendle & Telford, 1970). This method appears to cause only minimal alterations of physiological processes and it is unlikely that any regimen based on daily administration of drugs can produce greater freedom from side-effects while achieving absolute efficacy.

A new approach to the pharmacological control of human fertility is therefore necessary and an antagonist of progesterone would appear to be advantageous as this hormone is essential for, and relatively specific to, the maintenance

of pregnancy. It was therefore of interest to study a new steroid, RMI 12,936 (17 β -hydroxy-7 α -methylandrost-5-en-3-one: Text-fig. 1), which was claimed by its discoverers (U.S. Patent 3,833,621 issued 3 September 1974) to possess marked antiprogestational activity. Because oestrogens also show limited antiprogestational activity, some comparisons with a standard oestrogen, ethinyloestradiol, were also made.

MATERIALS AND METHODS

General

The SPF-derived rats of the Porton strain were housed at a temperature of 72°F $\pm$ 2°F with artificial illumination from 08.00 hours to 20.00 hours daily. The FFG/LAC diet and tap water were freely provided. Steroids were suspended in an aqueous vehicle containing 0.25% sodium carboxymethylcellulose (w/v) and 1% 'Tween 80' (w/v). Doses were administered subcutaneously in a dose volume of 0.5 ml except where otherwise stated. All surgical procedures were performed under light ether anaesthesia.

The day on which spermatozoa were found in the vaginal lavage was designated Day 1 of pregnancy.

TEXT-FIG. 1. The structure of RMI 12,936.

Vaginal cornification activity

Mature female rats were bilaterally ovariectomized, rested for 10 days, and checked for completeness of ovariectomy by the absence of cornified cells in vaginal lavages taken daily for 4 consecutive days.

The sensitivity of the animals to oestrogen was then checked by the subcutaneous administration of 5 μ g oestradiol dissolved in 0.1 ml of a mixture of 20% ethyl oleate (v/v) and 80% arachis oil (v/v) to each animal, followed by a further examination of vaginal lavages for 4 consecutive days. Only animals responding with fully cornified cells in the vaginal lavages on at least one occasion were subsequently used. The animals were then rested for a further period of 10 days and the various doses of RMI 12,936 and ethinyloestradiol were administered to groups of ten rats at each dose level. A further group was given the vehicle as a control. Vaginal lavages were again examined daily for 4 consecutive days and the number of animals responding with fully cornified smears was recorded.

Antifertility activity

Various dose levels of the compounds under examination were administered

to groups of ten female rats on Day 1 of pregnancy, while further groups of animals were dosed on Day 8. The animals were killed on Day 15, their ovaries removed and weighed to the nearest 0.1 mg, and the contents of their uteri examined. Pregnancy was considered to have been prevented if no live fetuses were found *in utero* at autopsy.

Effect on ovarian weight

One group of ten females was given 2 mg RMI 12,936 on Day 8 of pregnancy and a similar group was given the vehicle alone. A further group of ten animals was hysterectomized on Day 8 and given 2 mg RMI 12,936 while a similar group was given the vehicle alone. Ten mature cyclic females were also dosed with 2 mg RMI 12,936 and those in a further group were given the vehicle alone.

All the animals were killed 2 days after dosing and their ovaries were removed and weighed to the nearest 0.1 mg. The mean ovarian weights were calculated for each group and the significance of difference of means between each test group and the appropriate control was determined using Student's *t* test.

Effect of progesterone implants

A group of eight rats was given 2 mg RMI 12,936 on Day 7 of pregnancy while a further group of eight animals was ovariectomized. At the time of ovariectomy or dosing, each animal was given a subcutaneous implant of progesterone weighing approximately 25 mg and formed by cold compression. Further implants were inserted subcutaneously on Days 10 and 13. Rats of a control group were ovariectomized but not given progesterone implants. The animals were killed on Day 16 and the contents of their uteri were examined. Pregnancy was considered to have been maintained if any live fetuses were present *in utero*.

Effect on egg transport

A group of five rats was given 2 mg RMI 12,936 on Day 1 of pregnancy while a second group was given the vehicle as a control. Rats in a third group were subjected to laparotomy and a cotton ligature was tied round the base of each uterine horn. The animals were then given 2 mg of the steroid. At autopsy on Day 2, the oviducts were straightened by dissection and examined microscopically for the presence of eggs. The uterine horns were flushed with 0.5 ml normal saline and the washings were examined for the presence of eggs.

Further groups of five rats were given 2 mg RMI 12,936 at 6, 12 or 18 hr before autopsy on Day 2 of pregnancy when the positions of eggs within the reproductive tract were determined as described above.

Effects on egg viability

Pseudopregnant recipients were prepared by mechanical stimulation at 12.00 hours on the day of oestrus of the cervix of mature cyclic females with an engraving tool (Vibratool-Burgess) fitted with a smooth glass rod.

Fertilized eggs were obtained from pregnant animals given 2 mg RMI

12,936 on Day 4 and from animals given the vehicle as a control. Immediately before dosing, cotton ligatures were placed around the base of each uterine horn to prevent the loss of eggs from the reproductive tract of the animals treated with steroid and as part of the control procedure in those given the vehicle. Donor rats were killed on Day 5 of pregnancy and their uterine horns were flushed with sterile 0.5% NaCl solution (w/v). Recovered blastocysts were transferred to the uteri of 5-day pseudopregnant recipients through a mid-ventral incision at laparotomy. Five recipients received a total of forty-six eggs from donors treated with steroid while a further five received a total of forty-five eggs from control donors. The recipients were killed on Day 15 of pregnancy and the numbers of implantation sites and live fetuses present were recorded.

In a further control experiment, five animals were given 2 mg RMI 12,936 on Day 4 of pregnancy, five had cotton ligatures placed around the base of each uterine horn on Day 4, and five were given the steroid and had their uteri ligated. The animals were killed on Day 15 and the contents of the uteri were examined.

Effect on parturition

A group of five animals was given 2 mg RMI 12,936 on Day 19 of pregnancy while a similar group of five animals was given the vehicle only. All the animals were caged individually and checked twice daily to determine the time of parturition and the litter size for each animal.

Antifertility activity by the intravaginal route

Foam rubber tampons, approximately 10 mm in diameter and 15 mm long, were prepared by dissolving the required dose of RMI 12,936 in 1 ml warm ethanol, absorbing the solution into the tampon and then allowing drying to occur in air at room temperature. The tampons were inserted intravaginally with the aid of a small glass speculum on Day 8 of pregnancy. Five rats were used at each dose level and the control group was given blank tampons. The animals were killed on Day 15 and the contents of the uteri were examined. Pregnancy was considered to have been prevented if no live fetuses were present.

RESULTS

Vaginal cornification activity

The results given in Table 1 show that RMI 12,936 and ethinyloestradiol both induced vaginal cornification in the mature ovariectomized rat. Comparison of the minimal 100% effective doses showed that ethinyloestradiol was 10,000 times as active as RMI 12,936 in this test.

Antifertility activity

The results given in Table 2 show that RMI 12,936 and ethinyloestradiol caused termination of pregnancy in the rat after administration on Day 1 or Day 8. After administration on Day 1, RMI 12,936 was approximately one-tenth as active as ethinyloestradiol, but the two compounds were approximately

equipotent after administration on Day 8. Termination of pregnancy following administration of RMI 12,936 on Day 1 was associated with a highly significant decrease in ovarian weight at autopsy on Day 15, while administration on Day 8 caused highly significant ovarian hypertrophy.

Table 1. Relative vaginotrophic activities of RMI 12,936 and ethinyloestradiol in ovariectomized rats

<i>Ethinyloestradiol</i>		<i>RMI 12,936</i>	
<i>Dose (mg/rat)</i>	<i>No. of positive smears/ no. of treated rats</i>	<i>Dose (mg/rat)</i>	<i>No. of positive smears/ no. of treated rats</i>
0.0002	10/10	2	10/10
0.000064	1/10	0.64	1/10
0.00002	0/10	0.2	0/10
Vehicle only	0/10		

Table 2. Relative antifertility activities of RMI 12,936 and ethinyloestradiol in rats at autopsy on Day 15 of pregnancy

<i>Day of administration</i>	<i>Compound</i>	<i>Dose (mg/rat)</i>	<i>No. pregnant/ no. of treated rats</i>	<i>Mean wt of ovaries (mg)</i>
1	Ethinyloestradiol	2	0/10	81.4
		0.2	0/10	97.6
		0.02	9/10	109.6
	RMI 12,936	2	0/10	72.2*
		0.2	9/10	95.5
	Vehicle only	—	10/10	100.8*
8	Ethinyloestradiol	20	0/10	152.8
		2	0/10	90.2
		0.2	4/10	84.8
	RMI 12,936	2	0/10	133.2**
		0.2	7/10	101.4
		0.02	8/10	112.5
	Vehicle only	—	10/10	115.8**

* $P < 0.01$; ** $P < 0.01$.

Effect on ovarian weight

Ovarian hypertrophy in pregnant animals was apparent 2 days after dosing, (the mean wt of ovaries of control animals was 68.8 mg versus 80.6 mg in the treated rats, $P < 0.01$) but similar effects were not obtained in pregnant hysterectomized or non-pregnant animals. Between-experiment comparison showed that hysterectomy only and hysterectomy with RMI 12,936 treatment caused a similar degree of ovarian hypertrophy when compared to that in non-hysterectomized animals. Such comparison was not, however, valid as test and control animals were matched with respect to age and body size within, but differed between experiments, for which the body weight ranges were 160 to 200 g and 190 to 230 g, respectively.

Effect of progesterone implants

The results given in Table 3 show that a progesterone implant schedule capable of maintaining pregnancy in the ovariectomized rat was ineffective in animals treated with RMI 12,936.

Effect on egg transport

Administration of RMI 12,936 on Day 1 of pregnancy caused eggs normally located within the oviduct on Day 2 (a mean of 8.6 eggs in the control rats), to be prematurely transported to the uterus and expelled from the reproductive tract, except when the expulsion was prevented by ligation of the uterus (a mean of 9.2 eggs found in the uteri).

The effect on egg transport was not apparent 6 hr after dosing but had begun after 12 hr and was complete after 18 hr (Table 4).

Table 3. Effect of progesterone implants (25 mg) on maintenance of pregnancy in rats

Group	Days progesterone implanted	No. pregnant/ no. of treated rats	Mean no. of live fetuses
Ovariectomized, Day 8	—	0/8	0
Ovariectomized, Day 8	8, 11, 13	8/8	5.4
RMI 12,936 (2 mg/rat), Day 8	8, 11, 13	0/8	0

Rats were killed on Day 16.

Table 4. Effect of RMI 12,936 on the location of eggs at autopsy on Day 2 in rats

Time of dosing before autopsy (hr)	Mean no. of eggs/rat	Location of eggs
6	11.0	Isthmoampullary junction
	10.0†	Isthmoampullary junction
12	3.2	Isthmus
	9.0†	Isthmoampullary junction
18	0.2*	Isthmus
	9.4†	Isthmoampullary junction

The dose of RMI 12,936 was 2 mg/rat, there were five rats in each group and autopsy was on Day 2.

* These eggs were degenerating.

† Vehicle only.

Effects on egg viability

There was a reduction in, but not a complete inhibition of, egg viability following treatment of the donor with RMI 12,936 (48% transferred eggs implanting versus 78% in control rats, and a mean of 6.5% giving viable fetuses versus 35.5% in controls).

When RMI 12,936 was given on Day 4 of pregnancy, no viable fetuses were found, even when the uteri were ligated. Uterine ligation in rats receiving the vehicle only resulted in a mean of 4.8 viable fetuses.

Effect on parturition

Successful parturition was prevented in rats when RMI 12,936 was given on Day 19 of pregnancy (a mean of 0.6 dead fetuses/rat compared with 10.4 live fetuses in control rats). Expulsion of the dead fetuses and/or the products of fetal resorption occurred after a delay of 24 to 48 hr.

Antifertility activity by the intravaginal route

Pregnancy was terminated in all five rats after intravaginal administration of 10 mg RMI 12,936 on Day 8 of pregnancy.

DISCUSSION

The compound RMI 12,936 has been shown by J. O'N. Johnston, J. Greenwell, H. Benson and V. Petrow (unpublished data), and by preliminary experiments in our laboratories, to antagonize progesterone-induced endometrial proliferation in the rabbit. Oestrogens have similar effects and the present study was undertaken to investigate whether the compound was an oestrogen or whether it had specific antiprogestational activity. The results given in Tables 1 and 2 indicate that RMI 12,936, like ethinyloestradiol, can induce vaginal cornification in the ovariectomized rat and terminate pregnancy in the rat after administration on Day 1 or Day 8. The relative activities of RMI and ethinyloestradiol differed widely between tests, however, indicating major differences in the biological profiles of the two compounds. While RMI 12,936 can induce vaginal cornification and terminate pregnancy at the same dose level, the dose of ethinyloestradiol required to terminate pregnancy was approximately 1000 to 10,000 times higher than that required to induce vaginal cornification. One possible explanation for this finding is that RMI 12,936 and ethinyloestradiol have an affinity for both oestrogen and progesterone receptors in the reproductive tract. At oestrogen receptors the compounds would act as agonists and produce a response, while at progesterone receptors they would act as antagonists. Ethinyloestradiol would appear to have a high affinity for oestrogen receptors and a low affinity for progesterone receptors, while RMI 12,936 would seem to have an equal affinity for both receptor types. Since, however, RMI 12,936 fails to displace labelled progesterone from the cytosol progesterone receptor of human uteri (J. O'N. Johnston, personal communication), its antiprogestational activity is presumably mediated by interference with the activity of progesterone at some point other than this receptor.

The differences in ovarian weight following termination of pregnancy by administration of RMI 12,936 (Table 2) correlate with gross ovarian morphology at the time of autopsy. The highly significant reduction in ovarian weight after dosing on Day 1 is indicative of ovarian atrophy with neither corpora lutea nor large follicles present. The mechanism of antifertility activity following administration on Day 1 is an increased rate of egg transport resulting in a loss of eggs from the reproductive tract. The ovarian atrophy, however, suggests that, while the follicular suppression characteristic of pregnancy and pseudopregnancy is unaffected, the luteal development is blocked. Further experi-

ments are necessary to elucidate the mechanisms involved, but it is probable that the failure of luteal development is due to a lack of placental gonadotrophin following expulsion of the fertilized ova.

The observed ovarian hypertrophy following administration on Day 8 of pregnancy is associated with a visually apparent enlargement of the corpora lutea. This phenomenon may be confined to pregnant animals with intact uteri. If confirmed, this would indicate that the effect is probably mediated through increased production of placental gonadotrophin in response to a block of progesterone utilization by the products of conception. Since the fetal elements are rapidly destroyed by the drug, it seems likely that the source of the luteotrophic stimulus is the maternal placenta, as suggested by Holt & Ewing (1974). Further experiments are in progress to investigate this hypothesis, and to re-investigate the effects of RMI 12,936 and hysterectomy in a single experiment.

The antiprogestational effect of RMI 12,936 is due to inhibition of progesterone utilization rather than inhibition of synthesis or release, since pregnancy could not be maintained by a schedule of progesterone implants shown to be effective in the ovariectomized animal (see Table 3). These results are compatible with the hypothesis of competitive antagonism at the receptor level, with RMI 12,936 showing high affinity for progesterone receptors but low intrinsic activity at those sites. The biochemical findings with cytosol progesterone receptor from human uteri are evidence against this hypothesis.

It has been known for many years that oestrogens may increase the rate of egg transport, resulting in expulsion of eggs from the reproductive tract (see Greenwald, 1961). The dose required, however, is very much greater than that required to produce vaginal cornification (see Bennett, Kendle, Vallance & Vickery, 1966). Evidence in the rabbit (Chang, 1966; Kendle & Telford, 1970) indicates the importance of progesterone in the control of egg transport. It is therefore probable that the pharmacological effects of oestrogen are a reflection of their weak antiprogestational activity. This hypothesis would explain the activity of RMI 12,936 on egg transport at a dose level equivalent to that which produces vaginal cornification. Egg transfer experiments show that the most important site of action during the preimplantation period is probably on the maternal reproductive tract.

Because of the high antiprogestational activity of RMI 12,936, the compound appears suitable for development as a contraceptive for dosage at monthly intervals. Regular injections are undesirable as a means of fertility control but the present results show that the compound is effective when given intravaginally to rats. The compound has also been shown to be effective in rats at all stages of pregnancy. The side-effects of RMI 12,936 may also be reduced compared to those of available contraceptives due to the nature of its main pharmacological action and the much reduced frequency of administration.

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