Effect of RMI 12,936 on early pregnancy in mice

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Summary. Treatment with RMI 12,936 blocked the mitotic shift from epithelial to stromal cells normally observed in the uterus on Day 4 of pregnancy, and inhibited implantation. While progesterone alone could reverse the change in pattern of cell division in the preparation of the uterus for implantation, both oestradiol and progesterone were necessary to induce implantation and maintain pregnancy. A reduction in the activity of Δ^5 -3 β -hydroxysteroid dehydrogenase in the ovaries of RMI 12,936-treated mice suggests that the compound affects luteal synthesis of oestrogen and progesterone in mice.

Introduction

RMI 12,936 (17 β -hydroxy-7 α -methyl-androst-5-en-3-one) has been reported to possess anti-fertility activity when administered at any time during gestation in rats (Kendle, 1975, 1976, 1978; Saksena, Salmonsens & Lau, 1979b), hamsters (Lau & Saksena, 1979) and rabbits (Saksena, Lau & Chang, 1979a). In the rat, the compound caused an acceleration of egg transport and termination of pregnancy (Kendle, 1975), and was associated with a reduction in serum progesterone concentrations (Kendle, 1976) although no such reduction was found by Saksena *et al.* (1979b), who used a slightly different treatment schedule. However, Kendle & Lee (1980) have now shown that administration of RMI 12,936 inhibits egg transport in the mouse through an antiprogestational effect. It is suggested that some metabolite of RMI 12,936 may be acting as a competitive antagonist for progesterone at the receptor level, inhibiting progesterone synthesis in the ovary (Taylor & Kendle, 1978), or as an alternative substrate for Δ^5 -3 β -ketosteroid isomerase (Hardy, Kendle, Lawrie & Omand, 1977). The present study was undertaken to determine whether RMI 12,936 plays any role in the preparation of the uterus for implantation and subsequent pregnancy in the mouse.

Materials and Methods

The mice, weighing 20–25 g, were of the Swiss strain and of proven fertility. They were housed in air-conditioned rooms at a temperature of 21–24°C, humidity of 55–60% and 14 h light/24 h. The animals were fed on standard laboratory diet and water was supplied *ad libitum*. The females were caged with fertile males on the afternoon of pro-oestrus. The day on which a vaginal plug was observed was considered as Day 1 of pregnancy.

RMI 12,936 was suspended in an aqueous vehicle containing 0.25% sodium carboxymethyl cellulose and 1% Tween 80 (Kendle, 1975). The mouse dose was extrapolated from the rat dose

(Kendle, 1975), taking into consideration the surface area and body weight of the mouse. Progesterone and oestradiol were dissolved in olive oil.

Experiment 1. The effect of RMI 12,936 on mitotic cell division in the luminal, glandular and stromal cells of the mouse uterus was studied on Days 3 and 4 of pregnancy after arrest of mitosis by subcutaneous injection of 0.1 mg colchicine in 0.1 ml saline (9 g NaCl/l) 1 h before autopsy. Mice were treated with 0.6 mg RMI 12,936 on Days 1 or 2, on Days 1 and 2, or on Days 1, 2 and 3; some animals in the last group also received daily treatment with 1 mg progesterone. The numbers of cells showing mitotic activity were counted in 20 serial sections of the uterine horns of each animal, and the mean $\pm$ s.e.m. value was noted.

Experiment 2. On Day 4 of pregnancy, blastocysts were flushed from the uteri of mice treated with 0.6 mg RMI 12,936 on Days 1, 2 or 3. Some animals were also treated with 1 mg progesterone.

Experiment 3. Mice were injected with 0.3 or 0.6 mg RMI 12,936 at 10:00 h on Day 4 of pregnancy. To facilitate the detection of early implantation sites, 1% pontamine blue was injected intravenously before autopsy on Day 8. The effects of simultaneous hormone treatments were investigated by injecting RMI 12,936-treated mice with 1 mg progesterone daily from Days 4 to 20 or with 1 μ g oestradiol on Days 4 and 5 plus 1 mg progesterone daily from Days 4 to 20. The numbers of fetuses on Day 20 were recorded in other mice treated similarly.

Experiment 4. Animals were treated with 0.3 or 0.6 mg RMI 12,936 on Day 4 of pregnancy and were autopsied on Days 6, 7 or 8. Ovaries were weighed, frozen, and sectioned in a cryostat. They were histochemically stained for Δ^5 -3 β -hydroxysteroid dehydrogenase (Δ^5 -3 β -HSD) by the method of Dickmann & Day (1974), 17 β -hydroxysteroid dehydrogenase (17 β -HSD) by the method of Dey & Dickmann (1974) and β -glucuronidase (EC 3.2.1.31) by the method of Fishmann & Goldman (1965). Implantation sites were histochemically stained for alkaline phosphatase (EC 3.1.3.1) and acid phosphatase (EC 3.1.3.2) as described previously (Parthasarathy, Purandare, Katrak, Juneja & Munshi, 1979). Leucine aminopeptidase (EC 3.5.1.4) was stained by the method of Nachlas, Crawford & Seligman (1957). After staining the sections were mounted in glycerine jelly.

Results

Experiment 1. As shown in Table 1, the pattern of mitosis in mice in Groups 2–4 and 6 and 7 did not differ from that observed for the respective vehicle-treated control (Groups 1 and 5).

Table 1. Effect of RMI 12,936 (0.6 mg/mouse/day) on uterine cell division in mice (6/group) on Days 3 and 4 of pregnancy

Group	Treatment	Day of autopsy	Mean $\pm$ s.e.m. no. of mitotic figures		
			Epithelium		
			Luminal	Glandular	Stroma
1	None	3	10.5 $\pm$ 3.8	26.33 $\pm$ 4.3	1.0 $\pm$ 0.4
2	Day 1	3	8.5 $\pm$ 2.6	24.21 $\pm$ 3.2	1.2 $\pm$ 0.6
3	Day 2	3	8.0 $\pm$ 2.4	21.32 $\pm$ 2.4	1.4 $\pm$ 0.8
4	Day 1 + Day 2	3	8.3 $\pm$ 1.8	23.26 $\pm$ 2.8	1.0 $\pm$ 0.5
5	None	4	1.2 $\pm$ 0.8	1.6 $\pm$ 0.5	41.6 $\pm$ 4.5
6	Day 1	4	0	0	30.2 $\pm$ 3.1
7	Day 1 + Day 2	4	0	0	32.6 $\pm$ 2.3
8	Day 1 + Day 2 + Day 3	4	9.4 $\pm$ 1.2*	22.26 $\pm$ 4.2*	1.2 $\pm$ 0.1*
9	Day 1 + Day 2 + Day 3 + progesterone	4	1.0 $\pm$ 0.0	1.5 $\pm$ 0.0	40.25 $\pm$ 4.1

* Significantly different from Group 5 vehicle control, $P < 0.001$ (Student's t test).

However, RMI 12,936 administered on Days 1, 2 and 3 of pregnancy (Group 8) blocked the mitotic shift from epithelial to stromal cells observed on Day 4 of pregnancy. Simultaneous administration of progesterone (Group 9) overcame this inhibitory effect.

Experiment 2. Treatment with RMI 12,936 on Days 1, 2 and 3 of pregnancy reduced the number of blastocysts in the uteri on Day 4 (Table 2). Simultaneous progesterone treatment resulted in nearly normal blastocyst numbers.

Table 2. Effect of RMI 12,936 (0.6 mg/mouse) on the mean $\pm$ s.e.m. numbers of blastocysts in the uterus on Day 4

Treatment	No. of mice	No. of blastocysts
None	6	9.2 $\pm$ 0.2
Day 1	6	5.0 $\pm$ 0.2*
Day 2	7	4.0 $\pm$ 0.1*
Day 3	7	1.0 $\pm$ 0.1*
Day 3 + progesterone	6	8.4 $\pm$ 0.2

* Significantly different from the value in the vehicle control group, $P < 0.05$ (Student's *t* test).

Experiment 3. Implantation was inhibited by treatment with RMI 12,936 on Day 4 of pregnancy (Table 3). Administration of progesterone alone had no effect but progesterone + oestradiol resulted in normal implantation and maintenance of pregnancy.

Table 3. Effect of RMI 12,936 and steroids on implantation in the mouse

Treatment on Day 4			No. of mice with:	
RMI 12,936	Progesterone	Oestradiol	Implantation sites on Day 8	Fetuses on Day 20
—	—	—	6/6	6/6
0.3 mg	—	—	2/6	N.S.
0.6 mg	—	—	0/6	0/6
0.6 mg	1 mg*	—	0/6	0/6
0.6 mg	1 mg*	1 μ g†	6/6	6/6

* Injected daily Days 4 to 20.

† Injected only on Days 4 and 5.

N.S., not studied.

Experiment 4. Table 4 depicts the changes in the enzymes of ovaries and uteri of mice treated with RMI 12,936 on Day 4 of pregnancy. The activity of Δ^5 -3 β -HSD in the ovary and of alkaline phosphatase in the uterus decreased, but acid phosphatase and leucine aminopeptidase increased in activity. There was no effect on ovarian weights.

Discussion

During pregnancy, the change from epithelial to stromal mitosis occurs abruptly between Days 3 and 4, after exposure to progesterone for only 1 day (Finn & Martin, 1967; Martin & Finn, 1968). The uteri of mice treated with RMI 12,936 on Days 1, 2 and 3 failed to reveal the

Table 4. Effect of RMI 12,936 on mouse ovarian and uterine enzymes† on Days 6–8 of pregnancy

Treatment on Day 4	No. of mice	Ovary			Uterus		
		Δ^5 -3 β -HSD	17 β -HSD	β -glucuronidase	Alkaline phosphatase	Acid phosphatase	Leucine aminopeptidase
Vehicle	7	3.0 $\pm$ 0.1	1.5 $\pm$ 0.4	1.8 $\pm$ 0.2	4.0 $\pm$ 0.1	2.0 $\pm$ 0.1	2.0 $\pm$ 0.1
0.3 mg	8	2.7 $\pm$ 0.2	1.0 $\pm$ 0.1	1.3 $\pm$ 0.2	3.8 $\pm$ 0.2	2.1 $\pm$ 0.2	2.2 $\pm$ 0.1
0.6 mg	6	1.6 $\pm$ 0.2*	1.0 $\pm$ 0.1	1.6 $\pm$ 0.1	1.4 $\pm$ 0.2*	3.0 $\pm$ 0.1*	3.2 $\pm$ 0.1*

Values are mean $\pm$ s.e.m.

* Significantly different from the vehicle control value, $P < 0.05$ (Student's t test and Welch test).

† Estimated on a 4-point scale; 1.0, moderate; 2.0, good; 3.0, high; 4.0, intense.

cellular changes characteristic of Day 4 of pregnancy, but simultaneous administration of progesterone resulted in a normal appearance of the uterus. Our earlier studies (Munshi, Purandare & Rao, 1973) with LH antiserum treatment on Days 1, 2 and 3 also had the same effect which was completely reversed by progesterone alone. These results suggest that RMI 12,936 does not reduce endogenous oestrogen levels from Days 1 to 4 of pregnancy in mice, but it does interfere with the availability of progesterone from the corpus luteum.

Our results indicate that RMI 12,936 has an antifertility effect in mice by early expulsion of blastocysts from the uterus into the vagina. Simultaneous administration of 1 mg progesterone was able to induce normal mitosis and restore normal numbers of blastocysts in the uteri on Day 4 of pregnancy in RMI 12,936-treated animals. These results agree with the data obtained by Kendle & Lee (1980), indicating that progesterone activity is essential for the timely arrival of the blastocysts in the uterus on Day 4 of pregnancy in mice.

Our study also revealed that RMI 12,936 inhibits implantation in the mouse when administered during the preimplantation period. Characteristic morphological changes are evident at the time of implantation in the mouse (Munshi & Parthasarathy, 1979). In mice injected with RMI 12,936 on Day 4 and autopsied on Day 6 of pregnancy, vascularization and stromal cell proliferation was not seen. The absence of these morphological changes indicates that implantation had not occurred because implantation was histochemically reflected by a decrease in alkaline phosphatase activity and increase in acid phosphatase and leucine aminopeptidase activities in the uterus on Days 6, 7 and 8 of gestation. Continuous administration of progesterone alone was unable to overcome the inhibitory effect of RMI 12,936 on implantation, but oestradiol and progesterone were effective. Synthesis of progesterone and oestrogen in the ovary is dependent on the enzymic pathway which includes Δ^5 -3 β -HSD as one of the enzymes. Since the activity of this enzyme was decreased in the RMI 12,936-treated mice, the compound may be antioestrogenic as well as antiprogesterone at higher doses during implantation. A requirement for oestrogen on Days 6–8 (Exp. 3) confirms this view.

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