Induction of Preterm Birth in Mice by RU486


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ABSTRACT

We hypothesized that treatment of pregnant mice with the progesterone receptor antagonist RU486 might cause preterm labor and result in the delivery of live pups. We also hypothesized that RU486 administration would alter prostaglandin production by decidual explants taken from these pregnancies. C3H/HeN females mated with C57Bl/6 males were injected with a single s.c. dose of RU486 on Days 12-14 of gestation. Three doses were tested (50 μg, 150 μg, and 250 μg), and the mice were observed for evidence of delivery. The time course of delivery was determined in a second experiment using 150 μg of RU486, and care was taken to observe the condition of the delivered pups. In a third experiment, mice were killed when delivery commenced after injection with 150 μg of RU486, and decidual explants were prepared. Controls that had received injections of vehicle were killed at the same time, and decidual explants were prepared. Media were removed after 24 h and analyzed for prostaglandins E₂ (PGE₂) and prostaglandins F₂α (PGF₂α) by RIA and for interleukin 6 (IL-6) by ELISA. Two of 3 mice given 50 μg of RU486 delivered 16 pups prematurely. All 3 mice given 150 μg of RU486 delivered 22 pups prematurely, and 2 of 3 given 250 μg of RU486 delivered 12 pups prematurely. Of mice treated with 150 μg of RU486, none delivered within 12 h; 2 of 7 delivered within 15 h; and 6 of 7 delivered within 22 h. All pups appeared to be healthy, with no evidence of placental infarction or death. PGF₂α and IL-6 production by decidual explants was significantly greater in tissues taken from RU486-treated mice (n = 6) than in controls (n = 3). In summary, RU486 reliably induced preterm birth of the mice within 24 h after s.c. injection. This was associated with increased decidual prostaglandin and cytokine production and thus may mediate preterm labor. Inducing preterm birth with RU486 in a mouse model may be useful in investigations of the mechanism(s) of preterm labor.

INTRODUCTION

Preterm labor continues to be the leading cause of perinatal mortality and morbidity in the United States of America [1]. It is important to develop an appropriate and inexpensive animal model that can be manipulated experimentally in order to further our understanding of the underlying biochemical causes of this disease. Only then can rational new approaches be developed to improve treatment regimes. The mouse has a hemochorial placenta somewhat similar to human placentation, is small and relatively inexpensive, has a short gestational period, and can be easily manipulated experimentally. These factors have led us to develop a murine model for preterm labor focusing on infection as one cause of preterm birth. However, preterm birth in women has a multitude of causes, possibly including changes in circulating hormones.

One aspect of murine pregnancy is that term parturition is presaged by a precipitous decline in circulating serum progesterone concentrations [2, 3]. Although this does not happen in women, it is thought that a decrease in myometrial binding of progesterone does occur. Hence the final result of attenuated progesterone action is common to mice and women at term and would be mimicked by RU486 administration. In many studies it has been shown that acute reductions in progesterone production or action result in elevated rates of prostaglandin production [4-8]. Prostaglandins will induce labor in mice [9-12], and inhibition of prostaglandin biosynthesis can attenuate the actions of endotoxin to induce labor [9, 10]. Hence, the aim of this study was to determine whether the preterm pregnant mouse will respond to the progesterone receptor antagonist RU486 with labor and, if so, to characterize any changes in prostaglandin production in decidual tissue associated with the labor process. Additionally, we evaluated production of the cytokine interleukin 6 (IL-6) since we found that murine decidual explants produce inflammatory cytokines such as IL-6 after administration of lipopolysaccharide [13].

MATERIALS AND METHODS

Animals

Female C3H/HeN mice, aged 8-10 wk, were bred with C57Bl/6 male mice, and the day of vaginal plugging was designated Day 1 of pregnancy. All animal experimentation was performed according to the appropriate guidelines for animal use and was approved by the University of Utah Institutional Animal Care and Use Committee.

Experimental Design

On Day 12, 13, or 14 of pregnancy (term = 19-20 days), each pregnant mouse was treated with a single injection of RU486 (generous gift of Roussel-Uclaf, Romainville, France), which was dissolved to a concentration of 10-2 M in absolute ethanol, diluted in water, and administered in 1-ml volumes at one of three doses (see Table 1). The mice were closely observed for the onset of labor, as indicated by the onset of vaginal bleeding.

In the first set of experiments, the optimal route of RU486 administration was determined. In these experiments, RU486 was administered via i.p. injections or s.c. injections. The i.p. injections consisted of RU486 at the stated concentration diluted in 1 ml total solution. This amount was injected as a single bolus into the peritoneal cavity of each mouse. For the s.c. injections, 1 ml of total solution was injected in the s.c. region of the interior left or right flank of the hind leg of each animal.

Decidual Explants

In some experiments, decidual explant cultures were established after the RU486-treated mice entered labor (at the onset of bleeding). Saline-treated mice were killed by cer-
RU486 ACTIONS IN MICE

TABLE 1. Outcome following different doses and routes of administration of RU486.*

<table>
<thead>
<tr>
<th>Route and dose</th>
<th>n</th>
<th>Outcome</th>
<th>Fetuses/animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>No labor</td>
<td></td>
</tr>
<tr>
<td>RU486, 50 μg s.c.</td>
<td>3</td>
<td>2 delivered</td>
<td>5, 4, 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 undelivered</td>
<td></td>
</tr>
<tr>
<td>RU486, 150 μg s.c.</td>
<td>3</td>
<td>All delivered</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;24 h</td>
<td>8, 8</td>
</tr>
<tr>
<td>RU486, 250 μg s.c.</td>
<td>3</td>
<td>2 delivered</td>
<td>7, 7, 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;24 h</td>
<td>8, 4</td>
</tr>
<tr>
<td>RU486, 150 μg i.p.</td>
<td>3</td>
<td>1 undelivered</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;72 h</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No labor</td>
<td>5, 3, 8</td>
</tr>
</tbody>
</table>

* All animals administered 150 μg of RU486 s.c. delivered within 24 h of administration; all subsequent experiments used this dose and route of administration.

Results

The results of initial studies of treatments with various doses of RU486 are shown in Table 1. All pups were born alive. A dose of 150 μg of RU486 given s.c. caused delivery of all fetuses within 24 h; hence this dose and route of administration were used in all subsequent experiments. Interestingly, mice treated with i.p. injections had no preterm deliveries. Thus the route of drug delivery is an important component in this model. An evaluation of the time to delivery of the fetuses from mice treated with RU486 after the commencement of labor and delivery revealed that delivery occurred within 18 h for the majority (84%) of animals (Fig. 1). The remaining two animals were killed at 24 h and exhibited no signs of labor. Decidual tissue obtained from RU486-treated mice (n = 6) produced significantly more PGE2 than decidual tissue from saline-treated mice (n = 3) (Fig. 2). This effect was pronounced on Day 1 of culture but persisted through Days 2 and 3 of culture. Similar results were obtained for PGF2α production, although the effect was not statistically significant on Day 3 (Fig. 3). IL-6 production by decidual explants from RU486-treated animals was also significantly (p = 0.02) greater than IL-6 production by explants from saline-treated animals on Day 1 but not Days 2 or 3 of culture (Fig. 4).

Prostaglandin Assays

Prostaglandin E2 (PGE2) and prostaglandin F2α (PGF2α) in the culture supernatant were assayed by specific RIAs using antibodies obtained from Advanced Magnetics (Cambridge, MA), and culture medium was added to the supernatants in each culture supernatant were assayed by specific RIAs using antibodies obtained from Pharmingen (San Diego, CA). Intra- and interassay coefficients of variation were, respectively, 2.0% and 7.7% for PGE2 and 2.7% and 5.5% for PGF2α.

IL-6 Assay

Each culture supernatant was assayed for IL-6 by ELISA [15] using antibodies obtained from Pharmingen (San Diego, CA). Intra- and interassay coefficients of variation were, respectively, 4.5% and 7.1%.

Statistics

Differences between groups were determined using the Mann Whitney U test, with a significance level of ≤ 0.05.

FIG. 1. Time course of delivery after RU486 administration. Animals (n = 13) were administered RU486 (150 μg s.c.) and then observed for the onset of labor. This figure represents the cumulative percentage that delivered after RU486 administration across time. Two animals did not enter labor within 24 h of RU486 treatment, but most delivered within 15–18 h after RU486 administration.

FIG. 2. Production of PGE2 by decidual explants from mice treated with vehicle control (n = 3) or RU486 (n = 6). Decidual explants were isolated from mice treated with RU486 after the commencement of labor and delivery. Explants from controls were isolated at a concomitant time period. Culture supernatants were collected and assayed in triplicate for IL-6 production on each day of culture (see p values above columns).
This effect, however, was statistically significant only on Day 1 of culture.

**DISCUSSION**

The development and availability of RU486 as an active antiprogestational agent has provided not only many clinical applications but also many applications in biochemical and endocrinologic research as a tool to analyze the mechanisms of physiologic and pathophysiologic processes [16]. In this report, we have shown that RU486 treatment of pregnant mice in mid-gestation reproducibly results in preterm labor and delivery approximately 15–18 h after administration. Additionally, RU486 treatment of pregnant mice results in delivery of living fetuses and does not appear to mediate fetal death. This finding differentiates this method from others such as use of endotoxin [9, 10, 14], tumor necrosis factor (TNF) α [17], or IL-1 [18], although the latter may be equivocal [19].

Normal term labor in mice is anticipated by a significant drop in circulating progesterone levels [3], similar to that in other animal species such as sheep [5, 6]. Moreover, in women, decreased actions of progesterone on myometrium are noted during labor (see Introduction). Thus, antagonizing the effects of progesterone before term in mice similarly results in labor and delivery regardless of gestational age. Information on RU486 administration to rodents to study the mechanism of labor is limited. In a previous study by Potvin et al. [20], RU486 was used in rats to precipitate preterm labor and delivery in order to study the tocolytic effects of atrial natriuretic factor. In that study, RU486 administration at 16–18 days of rat gestation did not cause delivery within one to two days in half of animals treated. In our study, mice treated with s.c. RU486 rarely remained pregnant after 18 h of administration. Of note, mice treated with the i.p. injections of 150 pLg RU486 did not experience labor or delivery, suggesting that the route of delivery of the drug is critical for these effects. Sustained concentra-

![Graph](https://example.com/graph.png)  
**FIG. 3.** Production of PGF$_2$α by decidual explants from mice treated with vehicle control (n = 3) or RU486 (n = 6). Decidual explants were isolated from mice treated with RU486 after the commencement of labor and delivery. Explants from controls were isolated at a concomitant time period. Culture supernatants were collected and assayed as described (each value depicts mean ± SEM). There was significantly more production of PGF$_2$α by explants from RU486-treated mice on Days 1 and 2 of culture (see p values above columns).

![Graph](https://example.com/graph.png)  
**FIG. 4.** Production of IL-6 by decidual explants from mice treated with vehicle control (n = 3) or RU486 (n = 6). Decidual explants were isolated from mice treated with RU486 after the commencement of labor and delivery. Explants from controls were isolated at a concomitant time period. Culture supernatants were collected and assayed as described (each value depicts mean ± SEM). There was significantly more production of IL-6 by explants from RU486-treated mice on Day 1 of culture (see p values above columns).

We also found that administration of RU486 resulted in increased production of PGE$_2$ and PGF$_2$α by decidual explants. Our finding is similar to that of Jeremy and Dandona [21], who found that RU486 treatment of rats resulted in increased prostacyclin and thromboxane production by myometrial explants. In a similar study by Brooks et al. [22], RU486 treatment of pregnant rats resulted in increased production of decidual PGE$_2$ and decreased prostaglandin catabolism whereas RU486 administration to pregnant guinea pigs did not elicit increased decidual prostaglandin production. Also, Haluska et al. [23] found that treatment of Rhesus macaques at term with RU486 did not result in increased PGE$_2$ production by amnion. However, this group previously reported an increase in amniotic fluid prostaglandin concentrations in Rhesus macaques given RU486 in late gestation [24]. In humans, Cheng et al. [25] reported an increase in decidual PGE$_2$ production after RU486 administration in early pregnancy and suggested that part of the action of RU486 was to stimulate prostaglandin production through actions on 15-hydroxyprostaglandin dehydrogenase. Moreover, this effect appears to be more specific for human glandular cells and not stromal cells [7]. Thus, with regard to prostaglandin production, there are tremendous species-specific and tissue-specific differences in the responses to the actions of RU486. However, murine and human decidual preparations appear to have very similar responses to RU486 with regard to prostaglandin production.

The precise mechanism of increased IL-6 production by decidual explants is not known. The increase in IL-6 may be due to a direct stimulation by proinflammatory cytokines, such as IL-1 or TNF, or may be the result of alterations in progesterone-mediated regulation of the IL-6 gene. However, given the nature of this model, it is not possible to determine the exact biochemical mechanisms that led to the production of IL-6. Regardless, the results...
indicate that these explants produce cytokines in the process leading to preterm delivery. We have also found that the human decidual cells produce IL-6 in response to IL-1β and TNFα [15], leading us to speculate that human preterm birth is similarly associated with decidual production of IL-6.

Our findings that there is increased decidual prostaglandin and cytokine production by decidual explants after RU486 administration is strikingly similar to our previous reports [14], in which we found increased production of PGE2 and IL-6 by decidual explants after administration of lipopolysaccharide to pregnant mice at Days 12–14 of gestation. We have also found similar results after treating mice with TNFα and IL-1 [17, 18]. On the basis of these findings, we suggest that labor and delivery in mice, whether natural or induced, occurs along intermediate pathways that result in decidual production of cyclooxygenase products and inflammatory cytokines. While the stimuli for labor may differ, the mechanisms leading to labor appear to utilize basic common pathways. We believe that the mouse is a reasonable model for studying preterm labor in humans, as it is now recognized that preterm labor and delivery in humans probably represents a syndrome for which there are multiple etiologies [26]. In this regard, McDonald and Casey speculate that one key pathway to preterm labor is due to abnormalities in hormonal homeostasis [27] such as progesterone deficits or inadequate responses to progesterone. In our study, administration of RU486 to the pregnant mouse appears to be a reasonable model for analyzing the mechanism of hormonally mediated preterm labor and delivery. Thus, we believe that the murine model is a suitable choice for studying the heterogeneous mechanisms resulting in preterm birth.

REFERENCES