Uterine contractility and plasma levels of steroid hormones after intravaginal treatment of pregnant Japanese monkeys (*Macaca fuscata fuscata*) with 16,16-dimethyl-trans-Δ²-prostaglandin E-1 methyl ester

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Summary. Intravaginal treatment with a PGE-1 analogue (ONO-802) resulted in strong uterine contractions with a high frequency which lasted for more than 3 h. When 20–50 μg ONO-802/kg were administered intravaginally 5 times every 3 h in 5 pregnant animals, vaginal bleeding started by 6 h and abortion occurred by 26 h after the initial treatment. There were no significant side effects. Plasma levels of steroid hormones, especially progesterone and 20α-dihydroprogesterone, fluctuated initially and then finally dropped to undetectable levels within 24 h.

Introduction

The synthesis of prostaglandin (PG) analogues had provided highly potent therapeutic agents which stimulate uterine muscles but not other smooth muscles. Early studies with 16,16-dimethyl-PGE-2 free acid in the form of a vaginal solution revealed that the analogue exhibited undesirable gastrointestinal side effects (see Wiqvist & Martin, 1975), although Beguin, Bygdeman, Toppozada & Wiqvist (1972) suggested that the abortifacient effectiveness of the 15-methyl compounds but fewer of their side effects could be achieved with 16,16-dimethyl-PGE-2. The 16,16-dimethyl-trans-Δ²-PGE-1 methyl ester (ONO-802) does in fact have significantly decreased smooth muscle side effects and is 50–100 times more potent as a uterine muscle stimulant than PGF-2α when tested as a single intravenous injection in the non-pregnant monkey (Oshima, Matsumoto, Tsuda, Shibata & Hayashi, 1978).

The present study was therefore undertaken to determine the effectiveness of vaginally administered ONO-802 for the termination of pregnancy in Japanese macaques.

Materials and Methods

The Japanese monkeys (*Macaca fuscata fuscata*) used were from the Institute Colony and weighed 7–12 kg. The females were housed with 1 male from Day 11 to Day 15 of the menstrual cycle and pregnancy was verified by manual palpation of the uterus. The first day of pairing was considered to be Day 0 of gestation.

Uterine activity

In 3 monkeys at 50, 80 and 100 days of gestation, bipolar platinum electrodes were implanted at designated sites on the uterine surfaces as described previously (Oshima &

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Takenaka, 1977). The uterine myogram was recorded on a polygraph (Nihon-Koden 85M) simultaneously with internal pressure recordings obtained by a transabdominally implanted balloon catheter. The tracings were analysed for mean uterine activity, intensity and frequency of contractions and uterine tonus, after a single intravenous injection of 0·1–0·2 µg ONO-802/kg and 10–20 µg PGF-2α/kg. Each of the monkeys was also treated intravaginally with ONO-802 (20 µg/kg).

**Hormone changes**

Another 5 pregnant females were used to test the abortifacient effect of intravaginal treatment with ONO-802. Each monkey received 20–57 µg ONO-802/kg by intravaginal insertion of an agent-absorbed cotton ball every 3 h for 12 h, and at 24 h after the initial treatment. The total dose given to each animal ranged from 100 to 228 µg/kg. A solution containing 100–500 µg ONO-802 in 10% ethanol was prepared as the stock solution. Blood samples (3 ml) were withdrawn from 4 of 5 pregnant monkeys at 1, 2, 3, 6, 9, 12, 24 and 48 h after treatment and twice weekly before and after treatment for 1–3 months. Plasma samples were stored at -20°C until analysis.

Plasma levels of progesterone, 17α-hydroxyprogesterone, 20α-dihydroprogesterone, oestradiol and oestrone were determined by previously described radioimmunoassays involving purification of steroids on celite columns (Brenner, Guerrero, Cekan & Diczfalussy, 1973; Purvis, Brenner, Landgren, Cekan & Diczfalussy, 1975; Aedo, Landgren, Cekan & Diczfalussy, 1976). The validity of the assays for these steroids in the plasma of Japanese monkeys was investigated by the test of parallelism: increasing volumes of a pooled plasma sample (0·25, 0·5 and 1·0 ml) were compared with increasing quantities of authentic hormones, both in quadruplicate. Calculated F-values of parallelism and linearity for all steroids were not more than 3·15 and 2·67, respectively, while the tabulated F.95 was 4·54. The within-assay variation obtained from 20 measurements of a pool of plasma was 6·7–8·1%, and the between-assay variation, calculated from measurements of a plasma pool on 6 different occasions, was 9·3–13·1%. These results indicate that the assay methods yielded reliable results for the pregnant Japanese monkey. The sensitivities of the assays were 20 pg/tube for progesterone, 20α- and 17α-hydroxyprogesterone and 12·5 pg/tube for oestrone and oestradiol.

**Results**

**Uterine activity**

The results for all 3 animals were similar: a typical response is shown in Text-fig. 1. Intravenous injection of ONO-802 resulted in increased activity of the uterus (Text-fig. 1a). When ONO-802 was administered intravaginally, the rise in uterine activity occurred 3–6 min later (Text-fig. 1b). This rapid effect and the rise in uterine tonus suggests a quick vaginal absorption of the compound. Regular contractions with an amplitude of 20–30 mmHg followed about every 90 min. The repeated administration of 20 µg ONO-802/kg every 3 h maintained uterine activity until fetal expulsion.

**Hormone changes**

The abortifacient effects of ONO-802 are summarized in Table 1. There were no significant side effects except slight diarrhoea.

The changes of plasma concentrations of the five hormones studied varied with gestational age and individual. There was a pronounced fall in concentration of each of the 5 hormones, and particularly of progesterone, soon after initiation of ONO-802 treatment in Monkey 1 as shown in Text-fig. 2(a). A similar rapid fall was seen in Monkey 2, but the decline of plasma oestradiol...
Abortifacients in Japanese monkeys

Text-fig. 1. Recordings of electromyograms of the uterus and intrauterine pressure in a Japanese monkey at 100 days of gestation after (a) a single intravenous injection of 2 µg ONO-802/kg or (b) vaginal administration of 20 µg ONO-802/kg. The electrodes were implanted in the uterine fundus (U), uterotubal junction (UT) and uterine cervix (UC). The intrauterine pressure trace is indicated by IUP.

Table 1. Summary of the abortifacient effects of intravaginal treatment of pregnant Japanese monkeys with ONO-802

<table>
<thead>
<tr>
<th>Monkey no.</th>
<th>Body weight (kg)</th>
<th>Stage of pregnancy (days)</th>
<th>Dose (µg/kg) and frequency (every 3 h)</th>
<th>Time of onset of bleeding (h)*</th>
<th>Duration of bleeding (days)</th>
<th>Time of fetal expulsion (h)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.8</td>
<td>27</td>
<td>Day 27: 57 x 4</td>
<td>3</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>7.0</td>
<td>70</td>
<td>Day 70: 50 x 3</td>
<td>7</td>
<td>6</td>
<td>26.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 71: 50 x 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.0</td>
<td>80</td>
<td>Day 80: 20 x 4</td>
<td>6</td>
<td>6</td>
<td>27.5</td>
</tr>
<tr>
<td>4</td>
<td>6.4</td>
<td>90</td>
<td>Day 90: 20 x 4</td>
<td>3</td>
<td>—</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 91: 20 x 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5†</td>
<td>12.0</td>
<td>145</td>
<td>Day 145: 20 x 4</td>
<td>7</td>
<td>7</td>
<td>26</td>
</tr>
</tbody>
</table>

* From first treatment dose of ONO-802.
† Not used for hormone assay.
Text-fig. 2. Concentrations of oestradiol (○—○), oestrone (●...●), 17α-hydroxyprogesterone (▲—▲), 20α-dihydroprogesterone (●—●) and progesterone (○—○) in two Japanese monkeys (a, Monkey 1 at 27 days of gestation; b, Monkey 4 at 90 days of gestation) before, during and after intravaginal treatment with ONO-802 (a, 57 µg/kg at each dose; b, 20 µg/kg at each dose). Note the altered time scale in the stippled areas. The hatched bar indicates presence of the male, the solid bars periods and intensity of vaginal bleeding.
was the most marked. Oestradiol levels also increased in Monkey 3 about 21 h after the first treatment but the other hormones remained unchanged. In Monkey 4, which received a lower dose of ONO-802, plasma progesterone and 20α-dihydroprogesterone levels began to increase after the initial treatment, increased further after the third treatment, and then fell rapidly 21 h after the initial administration (Text-fig. 2b). The oestradiol values dropped slightly at first but were markedly increased by 21 h after the first vaginal treatment. Thereafter plasma levels of all five hormones declined.

Discussion

The present study confirms that 16,16-dimethyl-trans-Δ2-PGE-1 methyl ester is a potent stimulator of uterine muscle activity in pregnant monkeys. The effect of intravaginal administration on uterine contractility lasted approximately 3 h and an initial dose of 20 µg/kg followed by the same dose every 3 h might be an optimal treatment regimen, as reported by Martin et al. (1976). In all the animals which aborted, the mean abortion time was 26 h.

There were no severe gastrointestinal side effects associated with the vaginal administration of ONO-802 in the pregnant animals in this study, confirming our earlier electrophysiological findings (Oshima et al., 1978). The hormone studies also provided confirmation of the effectiveness of this agent. In the present experiments, the abortion was preceded by an elevation of tonic uterine contraction followed by rapid increase or decrease in plasma levels of progesterone and 20α-dihydroprogesterone, and final drop of all 5 steroid hormones. These changes of plasma concentration of steroid hormones may not result from a direct effect of ONO-802 on the corpus luteum, but may be caused by the strong uterine contractions leading to impaired function of the placental tissue. Since pregnancy can continue in the rhesus monkey after ovariectomy at 24 days after mating (Hodgen & Tullner, 1975) the abortifacient effect of ONO-802 is almost certainly due to its action on the uterine muscle in Japanese monkeys.

The clear surge of plasma oestradiol which occurred in 2 of the 4 animals 21 h after administration of the ONO-802 has also been seen in non-pregnant monkeys (unpublished data) and it therefore seems unlikely that elevation of plasma oestradiol is involved in abortions induced with ONO-802.

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References


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