Cloprostenol-induced luteolysis in the marmoset monkey (Callithrix jacchus)

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Summary. A single intramuscular injection of 0.5 µg cloprostenol was not luteolytic on Day 6 or 7 of the ovarian cycle (N = 3), but was luteolytic in some animals (3/5) on Day 8 and 9 and luteolytic in all 23 animals treated between Days 10 and 17 of the ovarian cycle, and in 7 animals treated between Days 19 and 43 of pregnancy. Luteal function was monitored by measurement of progesterone in peripheral blood using a simple and rapid non-extraction assay. There was a dramatic fall in peripheral blood progesterone to <10 ng/ml within 24 h of cloprostenol injection; progesterone remained at this low level until the day after post-treatment ovulation. The interval from cloprostenol injection to ovulation in animals treated between Days 8 and 17 was 10.7 ± 0.3 days. A similar interval was found in pregnant animals. Embryos recovered from the uterus after cloprostenol treatment were morphologically normal (23/24).

Introduction

The factors controlling the regression of the corpus luteum in primates are poorly understood. In many non-primates the luteolytic factor originates from the uterus and there is considerable evidence to support the concept that prostaglandin (PG) F-2α is the uterine factor responsible for luteolysis in laboratory and farm animals (Poyser, 1981). It seems unlikely that an identical mechanism is responsible for terminating the secretory activity of the primate corpus luteum in view of the failure of hysterectomy to prolong luteal function in the rhesus monkey (Neill, Johansson & Knobil, 1969) and man (Beling, Marcus & Markham, 1970). The hypothesis that endogenous PGs, possibly of ovarian origin, may nevertheless be involved in luteolysis in primates is supported by the findings that some exogenous PGs have luteolytic properties in man and monkeys (Korda, Shutt, Smith, Shearman & Lyneham, 1975; McCracken, Einer-Jensen & Fried, 1979; Vickery, McRae & Bajka, 1979; Wilks, 1983). However, other studies in which attempts to block PG synthesis have not prolonged luteal function do not support the involvement of PGs in primate luteolysis (Mannah & Novy, 1976).

As part of a series of studies directed towards understanding the control of the regression of the primate corpus luteum, we have examined the effect of the PGF-2α analogue, cloprostenol, on the fate of the corpus luteum of the common marmoset monkey (Callithrix jacchus). A secondary aim of the study was to develop a method to control the timing of ovulation in the marmoset.

Materials and Methods

Animals. The marmosets were housed in the Institute of Zoology primate colony as described by Harlow, Gems, Hodges & Hearn (1983) and 24 adult females with a mean body weight of 394.5 g (range 250–510 g) and regular cycles were used. They were housed with vasectomized males (N = 11) or in family groups with an intact male (N = 13). Ovarian cycles were monitored by radio-
immunoassay for peripheral blood progesterone. The day of ovulation (Day 0) was defined as the day preceding the rise of peripheral blood progesterone concentration above 10 ng/ml, and the last day of the luteal phase as the day preceding the drop of progesterone concentration below 10 ng/ml. Marmosets in our colonies have a mean ± s.e.m. cycle length of 28.6 ± 1.0 days with a luteal phase of 19.2 ± 0.6 days (Harlow et al., 1983).

**Experimental protocol.** Initially, several marmosets were treated with cloprostenol at doses ranging from 0.5 to 10 μg to determine a non-toxic dose. Thereafter, animals were treated with a single intramuscular injection of 0.5 μg cloprostenol (Estrumate: I.C.I., Macclesfield, U.K.: lot Nos HS12, PU11) from Days 6 to 17 of the ovarian cycle (Day 0 = ovulation) and from Days 19 to 43 of pregnancy. Cloprostenol was diluted in saline (9 g NaCl/l) on the day of use and injected in a volume of 0.1 ml. As controls, 3 animals were injected with 0.1 ml saline on Day 9 (N = 1) or Day 11 (N = 2). The day of cloprostenol injection was designated as Day 0; 0.2 ml blood was collected from the femoral vein into a heparinized syringe on Days 0, 1, 2, 4, 6, 8, 9, 10, 11, 12, 13, and 14, and the plasma stored at −20°C until assayed for progesterone. Once initial experiments had established the luteolytic effect of cloprostenol and the likely interval to ovulation, the blood sampling protocol was limited to Days 0, 1, 9, 10, 11, 12 and 13. In the later stages of the study, blood sampling was further limited to Days 0, 1, 10, 12 and 14.

Blood samples were taken throughout the post-treatment ovarian cycle from 7 animals to determine the length of the ovarian cycle and its luteal phase.

**Progesterone assay.** Progesterone in marmoset plasma was measured by a direct, non-extraction method, developed from the extraction assay described by Kendrick & Dixson (1983) and Harlow, Hearn & Hodges (1984). The origin and cross-reactivities of the antiserum used have been reported previously (Harlow et al., 1984). In brief, the method was as follows: 5 μl plasma were added directly to the assay tube and made up to 0.5 ml with buffer (0.1 M-phosphate buffer containing 0.01% sodium azide and 0.1% gelatin, pH 7.0). Doubling dilutions of progesterone standards were run over the range 400–625 pg/0.5 ml. Antiserum (100 μl) and tracer (100 μl or 10 000 c.p.m. [1,2,6,7-3H]progesterone; sp. act. 104 Ci/mmol: Amersham International Ltd, Bucks, U.K.) were added and the contents mixed and incubated at 37°C for 1 h and at 4°C for a further 30 min. Charcoal–dextran (0.625% and 0.0625%, respectively) was added in 200 μl buffer and the contents of the tubes were incubated for 15 min before centrifuging at 500 g and 4°C for 10 min.

Initially, danazol (17α-pregna-2,4-dien-20-yno[2,3-d]isoxazol-17β-ol), a gift from Winthrop Laboratories, Surbiton-upon-Thames, Surrey) was included in the assay as described by McGinley & Casey (1979) and Ratcliffe, Corrie, Dalziel & MacPherson (1982) to bind with and displace progesterone from any binding proteins present in marmoset plasma. Danazol used at concentrations of 0.16–40 μg/ml had no effect on the binding of 10 000 c.p.m. [3H]progesterone by 5 μl plasma. The use of danazol in the assay was therefore discontinued.

Serial dilutions of marmoset plasma (1 : 20–1 : 320) were tested for parallelism against standard curves prepared in assay buffer (as normal) and in buffer containing 5 μl charcoal-treated marmoset plasma/tube. There was no deviation from parallelism (P > 0.05, ANOVA) between the dilutions of marmoset plasma and either standard curve over the range 1 : 50 (20 μl) to 1 : 320 (3 μl) plasma. The procedure finally adopted was therefore based on the measurement of progesterone in 5 μl unextracted plasma without further modification to the assay described by Kendrick & Dixon (1983) and Harlow et al. (1984).

Progesterone values obtained by extraction and non-extraction assays were compared in a total of 44 plasma samples from 3 female marmosets. The results (Text-fig. 1) showed that there was a very good correlation between the two measurements over a range of values (1.7–121 ng/ml) likely to be encountered during the marmoset ovarian cycle. Accuracy was tested by the addition of various known amounts of progesterone standard (50–300 pg/tube) to a marmoset plasma pool containing low levels of progesterone. Mean ± s.d. recovery was 102.6 ± 4.9% (n = 18). Intra- and inter-assay precision, expressed as the coefficient of variation for repeated determinations of a
Text-fig. 1. Correlation of results for progesterone concentration obtained by conventional radioimmunoassay of extracted plasma and by direct radioimmunoassay of unextracted plasma from marmosets.

marmoset plasma pool, was 3.3% (n = 12) and 8.2% (n = 20) respectively. The sensitivity of the assay was 6.25 pg/tube (90% binding) or 1.25 mg/ml.

**Embryo collection.** Embryos were collected surgically from the uterus from 11 animals after cloprostenol treatment, using methods described by Hearn (1980). Embryos were evaluated morphologically for normal development and used in other experiments.

**Statistics.** The data were evaluated by two-way analysis of variance: a probability level of \( P < 0.05 \) was considered statistically significant.

**Results**

**Adverse physiological effects of cloprostenol**

Doses between 1.0 and 10.0 µg in preliminary experiments caused transient effects 2–10 min after injection, including retching, defaecation and rapid respiration. A dose of 0.5 µg cloprostenol did not induce any observable adverse physiological effects in any of the treated animals and this dose was adopted for all the work described below.

**Cloprostenol-induced luteolysis**

Cloprostenol was not luteolytic in 3 animals treated on Days 6 or 7 and progesterone concentrations remained elevated until at least Day 15–16 (Text-fig. 2). The animal treated on Day 7 was treated again on Day 15 and luteolysis occurred. In 2 of the 3 animals treated on Day 8, the dose of cloprostenol was luteolytic, but in the third animal, which was paired with an intact male, there was a transitory fall of peripheral progesterone to <10 ng/ml 24 h after treatment, although 24 h later progesterone was >40 ng/ml and remained above this level until Day 22 (Text-fig. 2). Cloprostenol treatment on Day 22 caused luteolysis in this animal. A transient drop in progesterone without permanent luteolysis also occurred in 1 of 2 animals treated on Day 9. This animal was paired with a vasectomized male and failure of luteolysis may be related to the fact that the treatment cycle was longer (34 days) than the average with a luteal phase in excess of 23 days. In all animals treated after Day 9 (23 animals, 33 treatments), there was complete and irreversible luteolysis within 24 h of treatment as indicated by the peripheral progesterone concentrations which fell to <10 ng/ml and
remained below this level until Day 1 of the next cycle (Text-fig. 2). Luteolysis was not induced in the 3 animals treated with saline only.

The interval (mean ± s.e.m.) from cloprostenol injection to ovulation, in 36 treatments between Days 8 and 17 inclusive and in which permanent luteolysis was induced, was 10.7 ± 0.3 days (range 9–13 days). The interval to ovulation for treatments between Days 8 and 12 (10.6 ± 0.2 days, N = 17) was not significantly different from that for treatments in the late luteal phase (Days 13–17) (10.3 ± 0.3 days, N = 19), nor was the interval to ovulation significantly influenced by whether females were paired with vasectomized or intact males.

Cloprostenol was also effective in inducing luteolysis in animals between Days 19 and 43 of pregnancy (Text-fig. 2): the interval from injection to ovulation was 10.9 ± 0.5 days (N = 7).

The post-treatment cycles monitored in 7 animals (10 treatments) were of normal length (27.6 ± 0.5 days) with a luteal phase of 18.7 ± 0.7 days. Of the 24 embryos (morulae to hatched blastocysts) collected on Days 6–9 after treatment of 11 animals, 23 were morphologically normal: 1 morula was degenerate.

Text-fig. 2. Peripheral blood progesterone concentrations in individual marmosets after treatment with cloprostenol on different days of the ovarian cycle and during pregnancy.
Discussion

These results clearly show that a single injection of 0.5 µg cloprostenol is able to terminate corpus luteum function effectively in the marmoset after Day 8 of the ovarian cycle and also during pregnancy. We conclude that because cloprostenol causes a rapid decline in peripheral blood progesterone concentration and it is equally effective in non-pregnant and pregnant animals, its action is likely to be directly on the corpus luteum rather than producing an increase in uterine contractility with subsequent embryonic death and termination of luteal function. Other workers have reported that some PGs and their analogues do have either transient or permanent luteolytic properties in certain laboratory primates, particularly rhesus monkeys, but in most cases are only effective when given as multiple injections, infusions or as vaginal suppositories (McCracken et al., 1979; Rall, Zuurmond & Weidemann, 1979; Wilks, 1983). However, Vickery et al. (1979) showed that a single injection of the PG analogue, prostalene, was effective during the mid- to late luteal phase in rhesus monkeys.

The inability of cloprostenol to cause luteolysis of the young corpus luteum of the marmoset is consistent with the observation made in a number of species that only the mature corpus luteum is susceptible to luteolysis by PGF-2α and its analogues (Poysen, 1981). It is not clear why young luteal cells are resistant to PGF-2α. There does not seem to be an increase in PGF-2α receptors on luteal cells during the transition from resistant to sensitive state and it has been suggested that there are other factors responsible apart from the receptor–ligand interaction per se (Wright, Pang & Behrman, 1980).

It is not clear whether the primate corpus luteum of the non-fertile cycle regresses due to the influence of an endogenous luteolysin or fails to survive because of the absence of luteotrophic support from the implating embryo. Since cloprostenol is luteolytic in the marmoset, it suggests, but does not prove, that endogenous PGF-2α might be the natural luteolysin in this primate. If this is the case, then endogenous PGF-2α would most likely be of ovarian origin because hysterectomy in the marmoset does not influence the life-span of the corpus luteum (Hearn, 1977). There is evidence that luteal cells are able to produce PGs in some primates (see Rothchild, 1981). Furthermore, Rothchild (1981) has proposed that intraluteal PGs are the final common agent of luteolysis in all mammals, and in primates the corpus luteum has remained the sole source of PGs, whilst in other species uterine PGs have been incorporated into the processes that initiate intraluteal PG production.

Our finding that the interval from luteal regression to ovulation was similar when marmosets were treated during the mid- or late luteal phase or during pregnancy is in accord with the view that follicular growth in primates is suppressed until the end of the luteal phase (Baird, Baker, McNatty & Neal, 1975; diZerega & Hodgen, 1981). This interval is also similar to the average time (10-5 days) from parturition in the marmoset until post-partum ovulation (Chambers & Hearn, 1979). Harlow et al. (1983) have described a follicular phase, as defined by the period in which peripheral progesterone levels are <10 ng/ml, of 8.3 ± 0.3 days. However, as our results show that an average interval of 10.7 days is required for growth of follicles to the ovulatory stage after the sudden termination of corpus luteum function with cloprostenol, it indicates that follicular growth begins during the last 2 days of the natural luteal phase, concomitant with a gradual decline in circulating progesterone as luteal function is ending.

The use of cloprostenol as an efficient, reliable and easily administered luteolytic agent for the marmoset is of major practical importance for experiments involving the collection and transfer of embryos. Furthermore, the collection of morphologically normal embryos following cloprostenol-induced luteolysis, and the fact that post-treatment cycles are of normal length, suggest that the endocrinological events of the post-treatment ovarian cycle are not affected by premature luteolysis.

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References


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