Termination of pregnancy and induction of premature luteolysis by the antiprogestagen, mifepristone, in dogs

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Summary. Five pregnant beagle bitches were treated with 2.5 mg mifepristone/kg body weight, twice a day, for 4.5 days starting at Day 32 of gestation. Results of fetal ultrasonography and assay of serum progesterone concentrations every 2–4 days were compared to those in 5 control bitches. Mifepristone resulted in a premature (P < 0.01) termination of pregnancy (36 ± 1 vs 65 ± 1 days), without side effects. The antiprogestagen also caused progesterone to decline to <1 ng/ml by Day 40–45 after the preovulatory LH peak (vs 64–67 days in controls) and reduced (P < 0.05) mean concentrations on Days 34–50 (2.2 ± 0.5 vs 6.3 ± 0.3 ng/ml). The results suggest that antiprogestagen therapy is a safe means to terminate unwanted pregnancy in dogs, and that luteal function in pregnant bitches is dependent on luteotrophic support that is blocked by antiprogestagen treatment, directly or indirectly, due to termination of pregnancy.

Keywords: progesterone; pregnancy; abortion; antiprogestone; corpus luteum; luteolysis; dog

Introduction

Methods for post-coital contraception and induction of abortion in dogs are needed to prevent the birth of unwanted litters that result from unplanned matings. However, safe and efficacious methods are not readily available and oestrogen administration to prevent implantation can have particularly harmful effects (Concannon, 1983; Bowen et al., 1985). Pregnancy in the bitch is dependent on ovarian progesterone secretion throughout gestation (Sokolowski, 1974; Concannon et al., 1977) and both LH and prolactin are required luteotrophins (Concannon et al., 1987). Pregnancy can be terminated by luteolytic treatments, including repeated administration of prostaglandin F-2α (Concannon & Hansel, 1977; Paradis et al., 1983) or of prolactin-lowering doses of bromocriptine (Concannon, 1986). Both treatments produce various undesirable side effects and neither is approved for use in dogs. The potential to terminate pregnancy in dogs by administration of an antiprogestagen has not been reported. Mifepristone, a 19-norsteroid [17β-hydroxy-11β-(4-dimethylamino-phenyl)-17α-(1-propynyl)-oestra-4,9-diene-3-one: Roussel-Uclaf, Paris, France] is a progesterone and glucocorticoid receptor antagonist. Its antiprogestagenic activity has been demonstrated in rodents and rabbits (Philibert et al., 1982), monkeys (Koering et al., 1986) and dogs (Concannon et al., 1988a). Anticorticoid activity has been demonstrated in rodents (Jung-Testas & Baulieu, 1983), man (Gaillard et al., 1985) and dogs (Spitz et al., 1985). Mifepristone has been reported to terminate pregnancy in monkeys (Hodgen, 1985) and women (Kovacs et al., 1984; Couzinnet et al., 1986). Administration of this antiprogestagen can also cause premature luteolysis in non-pregnant women (Nieman et al., 1987). The present study was conducted to determine the effects of low doses of mifepristone on pregnancy maintenance in dogs and potential concomitant effects on luteal function.
Materials and Methods

The bitches were 2-6-year-old beagles maintained indoors and monitored for pro-oestrus and oestrus as previously described (Concannon et al., 1977, 1987). Blood samples were collected and vaginal smears examined every 1-2 days during late pro-oestrus and oestrus. Serum progesterone was determined in assays conducted every 2-3 days to estimate the day of the LH peak and bitches were mated 2 or 3 times on alternate days beginning 0-3 days after progesterone rose above 1 ng/ml. Vaginal cytology was monitored until the late oestrous decline in superficial cells was observed and also used retrospectively to estimate the date of the preovulatory LH surge as occurring about 8 days earlier (Concannon & DiGregorio, 1986). The day of the preovulatory LH surge was considered to be the first day which was 7-9 days before the late-oestrous change in the vaginal smear and also within 1 day of the first elevation in progesterone above 1 ng/ml which was followed by a persistent rise in progesterone.

Pregnancy was confirmed by ultrasound at 20-25 days after the first mating in each of 11 bitches. Five bitches were assigned as untreated controls and 5 were treated with mifepristone, starting at Day 32 of pregnancy. Day 0 of pregnancy was the estimated day of the preovulatory LH surge. The mifepristone was put into gelatin capsules (No. 2410-62, Eli Lilly, Indianapolis, IN, USA) in amounts based on the body weights of the bitches at 1 day before start of treatment. Body weights ranged from 12 to 15 kg. The 5 mg/kg/day dose of mifepristone was given orally, twice daily (07:00 h and 19:00 h) at 2.5 mg/kg body weight. Treatment in each bitch lasted 4-5 days, being discontinued 0-1 days after ultrasound or clinical confirmation of obvious fetal wastage. One additional bitch was treated with mifepristone for 6-5 days, starting on Day 22 of pregnancy and the 1st day that fetal heart movements could be detected by ultrasonography, and ending on Day 28 when all but one of the fetuses were no longer alive. Ultrasound examinations at intervals of 1-3 days monitored fetal heart movements and fetal size until Day 40 of pregnancy. Bitches were observed daily for general health, appearance and activity. Progesterone was assayed in serum samples obtained every 2-4 days or more frequently from 3 days before treatment until Day 58-62 of pregnancy, or until parturition.

Blood samples (3-10 ml) were obtained via jugular venepuncture into evacuated tubes, allowed to clot for 12-18 h at 5°C and centrifuged at 1500 g. Serum aliquots were stored frozen until assayed. Progesterone in serum was measured in duplicate samples using a commercial solid-phase progesterone radioimmunoassay (Coat-A-Count Progesterone, Diagnostic Products Corp., Los Angeles, CA, USA) as described by Srikandakumar et al. (1986) for dog plasma and used in our laboratory (Concannon, 1989). In brief, 100 μl samples of dog serum, or standard progesterone in human serum were incubated in antibody-coated tubes along with 125I-labelled progesterone for 3 h at 22°C. The entire volume was aspirated, and radioactivity in the tubes was counted. Each assay also included aliquots of 2 serum pools which had been previously assayed for progesterone content using extraction, monitoring of recovery, antiserum of high specificity, and validated double-antibody radioimmunoassay procedures previously described in detail (Concannon et al., 1983; Reimers et al., 1983). The sensitivity was 0-1 ng/ml. Mean within-assay coefficients of variation ranged from 4 to 7% and averaged 6%. Between-assay coefficients of variation for 2 pools of serum containing 4 and 15 ng/ml were 8% and 6%, respectively, in 8 assays.

Results

The number of individual fetuses that could be distinguished and monitored by ultrasound varied within individual bitches and ranged from 4 to 7. However, the actual numbers may have been greater. The effects of mifepristone treatment starting at Day 32 are summarized and compared to observations in control pregnancies in Fig. 1. The absence of fetal heart movements in individual fetuses was first observed at 2-3-5 days after start of treatment. Complete termination of pregnancy, defined as the death of all fetuses, occurred 3-5-4-5 days after start of treatment on Day 32. However, in the bitch for which treatment started on Day 22 of pregnancy, the last fetus but one died after 6-5 days, and the remaining fetus died at 15 days after start of treatment (see Fig. 2).

Among bitches treated from Day 32, the expulsion of 4-6 fetuses from each was documented for 3 dogs at 4-6 days after start of treatment. In the other 2 bitches a dark mucoid discharge was observed beginning at the same time; expulsion of individual fetuses was not documented, ultrasound showed no evidence of retained or resorbing fetal masses, and aborted material may have been ingested. For the bitch treated from Day 22 no vaginal discharge was observed during treatment. No changes in general health, appearance or behaviour were observed in any of the treated bitches.

Serum progesterone concentrations in each antiprogestagen-treated bitch declined to < 1 ng/ml between 35 and 45 days after the preovulatory LH surge. Progesterone values were maintained above
Fig. 1. Mean (± s.e.m.) serum progesterone concentrations and times of parturition or abortions for 5 control pregnant beagle bitches and 5 bitches treated with mifepristone at an oral dose of 2·5 mg/kg twice a day for 4·5 days starting on Day 32 of pregnancy. Standard error bars are not indicated where they are smaller than the symbol for the means.

Fig. 2. Serum progesterone concentrations in a control pregnant beagle bitch and in 2 bitches treated with mifepristone (2·5 mg/kg twice daily for 4·5 days), one from Day 32 of pregnancy and aborting on Day 36, and one from Day 22 of pregnancy and aborting 5 fetuses by Day 28 and aborting a remaining single fetus on Day 37.

2 ng/ml until Day 62 in control pregnancies and did not decline to < 1 ng/ml until parturition on Day 64–67, or shortly thereafter (Fig. 1).

The mean serum concentrations of progesterone after treatment (Days 34–50) ranged from 1·3 to 4·9 ng/ml in bitches treated with mifepristone from Day 32, and from 4·2 to 9·2 ng/ml in controls. Mean progesterone values in bitches administered mifepristone (2·2 ± 0·5 ng/ml) were lower (P < 0·05) than in controls (6·3 ± 0·3 ng/ml).
Discussion

The results suggest that oral antiprogestagen therapy can be used as a safe and effective method for the termination of unwanted pregnancy in dogs. The rapid termination of pregnancy by mifepristone treatment is consistent with the reported progesterone-antagonist effects of this steroid in several species, including dogs (Concannon et al., 1988a), monkeys (Healy et al., 1983; Koering et al., 1986) and humans (Schaison et al., 1985), and the ability of mifepristone to terminate pregnancy in women (Kovacs et al., 1984; Couzin et al., 1986). The fact that the termination of all pregnancies occurred in the presence of elevated concentrations of progesterone demonstrates that the abortifacient effect was due to direct progesterone antagonist effects at the level of the uterus and independent of any additional effects on luteal function.

The premature cessation of luteal function in antiprogestagen-treated animals may have occurred secondary to the termination of pregnancy or may represent a luteolytic effect of treatment independent of pregnancy status. There is some evidence that pregnancy in dogs promotes greater luteal activity and progesterone secretion than that observed in non-pregnant dogs at a comparable time in the luteal phase (Smith & McDonald, 1974; Concannon et al., 1977). Perhaps some pregnancy-specific luteotrophic activity was withdrawn by termination of pregnancy in these bitches. Luteolysis secondary to abortions or resorptions could be related to a uterine release of luteolytic amounts of prostaglandin F-2α as such normally occurs at parturition (Concannon et al., 1988b). Mifepristone can induce endometrial prostaglandin secretion in vitro (Kelly et al., 1985). A pituitary effect could also be involved, since mifepristone has antigonadotrophic activity in non-pregnant women (Schaison et al., 1985). Finally, a direct ovarian effect cannot be dismissed. Mifepristone suppresses 3β-hydroxysteroid dehydrogenase and 17-hydroxylase activities of human ovarian tissue in vitro (DiMattina et al., 1987).

A longer period of treatment with mifepristone was required to compromise most of the fetuses when started at Day 22, when serum progesterone concentration at the start of treatment was higher than in bitches treated later in pregnancy. This suggests that the 5 mg/kg dose of mifepristone may have been near the lower end of the effective range, and that abortifacient efficacy may be dependent on the serum concentrations of progesterone present during treatment. Such a conclusion would be in agreement with the fact that the 5 mg/kg dose antagonized only about 60% of the uterine stimulation caused by physiological doses of progesterone in oestrogen-primed immature bitches (Concannon et al., 1988a). In contrast, a dose of 20 mg/kg antagonized over 80% of the activity of the exogenous progesterone. Oral antiprogestagenic activity of mifepristone in dogs may be lower than in humans, since in women a single dose of 10 mg/kg consistently induced menses during the mid-luteal phase when plasma progesterone concentrations were 8–34 ng/ml.

Since no adverse side effects were noted, increasing the dosage to 10 mg/kg/day might be an appropriate means to increase abortifacient efficacy during early pregnancy. In humans, abortifacient efficacy had no apparent dose dependency in a study with a narrow dose range (Couzin et al., 1986), but the luteolytic effect did appear to be dose-dependent (Nieman et al., 1987). The latter may be true in dogs. Parenteral doses of 2 mg/kg at 2–3-day intervals reduced the extent of clinical pseudopregnancy in bitches but did not significantly shorten the lifespan of the corpus luteum (Gerres et al., 1988). Although mifepristone has antianticorticoid activity in dogs (Spitz et al., 1985), antianticorticoid activity was limited to doses of 20 or 50 mg/kg and not observed with the 5 mg/kg dose, as used in the present study. In conclusion, administration of an antiprogestagen such as mifepristone terminates pregnancy in dogs; the resulting luteolysis suggests a possible luteal dependence on fetoplacental integrity and/or a delayed sensitivity to pituitary, uterine or ovarian effects of antiprogestagen therapy.

We thank A. Montanez, M. Battista, M. Ullmann and B. Blinn for technical assistance, and G. Grossman and R. McGuire for care of the animals. This research was supported, in part, by grants...
from the Morris Animal Foundation and the New York State College Veterinary Medicine Alumni Fund.

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Received 27 February 1989