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CDB-2914: Anti-progestational/anti-glucocorticoid profile and post-coital anti-fertility activity in rats and rabbits*

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Our goal was to determine the endocrine and post-coital anti-fertility activity of CDB-2914. Concurrent administration of progesterone to rats on day 4 post-mating blocked the anti-fertility activity of a single oral 2 mg dose of CDB-2914. CDB-2914 did not exhibit progestational activity in the oestradiol-primed immature female rabbit at doses that exhibited anti-progestational activity. CDB-2914 antagonized exogenous and endogenous progesterone-stimulated uterine haptoglobin synthesis and secretion in immature and adult mated rabbits respectively. Neither CDB-2914 nor mifepristone exhibited glucocorticoid activity as determined by thymus involution in rats; mifepristone was twice as potent as CDB-2914 in antagonizing glucocorticoid action. Post-coital CDB-2914 treatment resulted in a dose-dependent reduction in implantation sites and pregnancy rates in rabbits. CDB-2914-induced inhibition of uterine weight increase, endometrial glandular arborization and uterine haptoglobin synthesis/secretion correlated with inhibition of pregnancy in mated rabbits. A single oral dose of 64 mg CDB-2914/rabbit was effective at blocking pregnancy when administered on day 4, 5, or 6 post-mating, whereas 32 mg/rabbit was only partially effective in this regard. These data demonstrate that CDB-2914 is a potent, orally active anti-progestin with weak anti-glucocorticoid activity. CDB-2914 inhibited implantation in adult rats and rabbits demonstrating its potential as a post-coital contraceptive drug.

Key words: agonist/antagonist/anti-fertility/glucocorticoid/progestin

Introduction

Several 11β-aryl substituted progesterone antagonists or anti-progestins have been synthesized and characterized in the past decade. Mifepristone was the first steroid of this chemical class shown to bind the progestin receptor (PR) with high affinity (Neef *et al.*, 1984; Kloosterboer *et al.*, 1988; Spitz and Bardin, 1993; Teutsch and Philibert, 1994; Spitz *et al.*, 1996; Cadepond *et al.*, 1997). The 11β N,N-dimethylaminophenyl moiety of mifepristone and other steroidal anti-progestins is believed to confer antagonist activity via interactions in the 11β-pocket of the PR resulting in the inhibition of transcriptional activity (Gronemeyer *et al.*, 1992; Cadepond *et al.*, 1997). The majority of these 11β-aryl substituted anti-progestins also bind the glucocorticoid receptor with high affinity and exhibit anti-glucocorticoid activity (Neef *et al.*, 1984; Gagne *et al.*, 1985, 1986; Busso *et al.*, 1987). In certain test systems, mifepristone and other anti-progestins have also exhibited progestational activity as well as weak anti-oestrogenic and anti-androgenic activity (Meyer *et al.*, 1990; Hodgen *et al.*, 1994; Slayden and Brenner, 1994; Hackenberg *et al.*, 1996). Importantly, anti-progestins have shown considerable potential in fertility control and in reproductive medicine such as in the treatment of breast cancer, endometriosis, and leiomyomata (Spitz and Bardin, 1993; Spitz *et al.*, 1996; Cadepond *et al.*, 1997). Presently, the only clinically approved anti-progestin is mifepristone which is administered acutely to induce medical abortions (Baulieu, 1991). The acute administration of mifepristone circumvents undesirable anti-glucocorticoid effects. For chronic or persistent disease states, a potent orally active compound with relatively pure anti-progestational activity is needed.

Currently, newer anti-progestins are being designed, synthesized, and tested in an attempt to identify a relatively pure progesterone antagonist. A promising 11β-aryl substituted, 17α-acetoxy progesterone analogue, CDB-2914, has been shown to bind with high affinity to the progestin and glucocorticoid receptor, and antagonizes progesterone action in the immature female rabbit with greater potency than mifepristone when administered orally (Cook *et al.*, 1992, 1994). In addition, CDB-2914 demonstrated potent anti-ovulatory and post-coital anti-fertility activity in the rat indicating its potential use as a post-coital contraceptive (Reel *et al.*, 1998). Anti-progestins have also been shown to have contraceptive potential in female rhesus monkeys when administered at a low chronic dose (Zelinski-Wooten *et al.*, 1998; Slayden *et al.*, 1998). The ovarian cycle was not affected in these monkeys; however, endometrial atrophy was apparent. Likewise, CDB-2914 was shown to suppress pregnancy in the rat when administered over a 24 day interval at a low daily dose (Reel *et al.*, 1998). Although CDB-2914 did not have detectable androgenic or oestrogenic activity, CDB-2914 did demonstrate weak anti-androgenic and anti-oestrogenic activity in immature male and female rats respectively (unpublished data). The objective of the present study was to confirm and extend the biological profile of CDB-2914 (also referred to as RTI 3021–012,

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RU44675, and HRP 2000 in other publications; Cook *et al.*, 1992, 1994; Teutsch and Philibert, 1994; Tarantal *et al.*, 1996; Wagner *et al*., 1996, 1999). In particular, the anti-progestational and anti-fertility activity of CDB-2914 in the rabbit model was assessed and the anti-glucocorticoid activity of CDB-2914 and its potency relative to mifepristone in an in-vivo model was investigated to determine whether CDB-2914 might have fewer potential side-effects in clinical use. CDB-2914 was reported to have 100-fold less anti-glucocorticoid activity than mifepristone in an in-vitro assay in which the ability of the anti-progestin/ anti-glucocorticoid to inhibit dexamethasone-stimulated transcriptional activity was examined (Wagner *et al.*, 1999). The relevance of these in-vitro data to in-vivo anti-glucocorticoid activity is not known at present.

Materials and methods

Animals

New Zealand White (NZW) rabbits [ILAR Strain Designation Hra:(NZW)SPF] were purchased from Covance Research Products (Denver, PA, USA) and housed in stainless steel cages. Rabbits were fed Purina (St Louis, MO, USA) laboratory rabbit diet (no. 5321 or 5326) and fresh kale daily as a dietary fibre supplement. Sprague-Dawley CD rats [Crl:CD(SD)IGS BR Stock] were purchased from Charles River Laboratories (Kingston, NY, USA) and group-housed in polycarbonate solid floor cages with Bed-o-Cob® or Beta-Chip® bedding (Andersons Industrial Products Group, Maumee, OH, USA) and were fed Purina laboratory rodent diet (no. 5001) *ad libitum*. All animals received tap water *ad libitum*, except for adrenalectomized male rats which received 0.9% saline *ad libitum*. The photoperiod for the rabbit rooms was 12 h light/12 h dark and for the rat rooms was 14 h light/10 h dark. The environmental conditions of the animal rooms were maintained as recommended in the *Guide for the care and use of laboratory animals* (National Research Council, 1996) to the maximum extent possible. All study protocols were approved by BIOQUAL's institutional animal care and use committee.

Materials

For these studies, CDB-2914, 17α-acetoxy-11β-[4-N,N-dimethyl-aminophenyl]-19 norpregna-4,9-diene-3,20-dione, was synthesized by P.N.Rao, Southwest Foundation for Biomedical Research, San Antonio, TX, USA, under contract N01-HD-1–3137. The batch designated as CDB-2914P was used for all assays except for the glucocorticoid/anti-glucocorticoid bioassays which used the batch designated CDB-2914R-2, and the reversal of post-coital anti-fertility activity which used the batch designated as CDB-2914U. All batches were synthesized using the same procedure, were 99% pure based on high-performance liquid chromatography (HPLC) analyses, and had similar potency in the immature rabbit anti-progestational bioassay (data not shown). Mifepristone was provided by Roussel-UCLAF, Romainville, France and was 99% pure based on HPLC analysis. Levonorgestrel was obtained from Schering AG (Berlin, Germany). Other steroids were purchased from Steraloids Inc., (Wilton, NH, USA). Needles, syringes, anaesthetic, and surgical supplies were purchased from A.J. Buck and Son Inc. (Owings Mills, MD, USA). Reagent grade chemicals were purchased from VWR Scientific Products Inc. (West Chester, PA, USA) or Sigma Inc. (St Louis, MO, USA). Food grade sesame oil (Hain) was purchased from a local grocery store (Giant, Gaithersburg, MD, USA).

Progestational/anti-progestational assays

Reversal of CDB-2914 post-coital anti-fertility activity with progesterone in the rat

In order to determine whether CDB-2914 post-coital anti-fertility activity was reversible and due primarily to antagonism of progesterone action, confirmed mated (day 0 = presence of vaginal spermatozoa or copulatory plug) female rats received a single oral dose of 2 mg CDB-2914/rat at 0930 h on day 4 post-mating. Progesterone (10 mg/rat/injection) was also administered s.c. at 0730 and 1530 h on day 4 post-mating. Female rats were killed on or about day 17 post-mating and the condition and number of implantation sites were determined.

Stimulation or inhibition of endometrial glandular arborization in the immature female rabbit

For the progestational bioassay, immature female rabbits were primed with 17β-oestradiol for 6 days and then treated orally with either the control vehicle, CDB-2914, or levonorgestrel for 5 consecutive days. Twenty-four h following the final dose, rabbits were killed, the uteri excised, trimmed of extraneous tissue, blotted, and weighed (Elton and Edgren, 1958). Sections (5 μm) of fixed uteri were evaluated for endometrial glandular arborization based on the scoring system of McPhail (McPhail, 1934). The anti-progestational bioassays were similar to the progestational bioassay, except rabbits received 0.16 mg progesterone/rabbit/day s.c. concurrently with control vehicle or CDB-2914 orally.

Induction and antagonism of uterine haptoglobin synthesis and secretion in the immature female rabbit

Uteri were also collected from rabbits primed with 17β-oestradiol for 6 days (n = 3), and 17β-oestradiol rabbits treated subsequently with progesterone concurrently with either control vehicle or increasing doses of CDB-2914 orally for 5 days (n = 5/group). After removing the 2 cm utero-tubal segment for fixation, the cut ends of the uteri were clamped with haemostats and the uterine horns infused with 2–3 ml per horn of Tris-buffered saline containing protease inhibitors (TBS-PI; Hoffman *et al.*, 1996) and the cervical end of the uterine horns clamped with another haemostat. Uteri were incubated 10–15 min, the incubation fluid collected, centrifuged at 500 *g* for 10 min to remove cells and debris, and the supernatant fraction frozen and stored at –70°C (Hoffman *et al.*, 1996).

Protein concentration was determined in the uterine incubation fluid using the Bradford method (Bradford, 1976) and samples diluted in electrophoresis sample buffer (Laemmli, 1970) lacking β-mercaptoethanol and loaded (10 μg protein/lane) onto 12% acrylamide gels. Non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent transfer to nitrocellulose membranes were completed as previously described (Hoffman *et al.*, 1996). A standard sample of uterine incubation fluid from a 6.75 day pregnant rabbit was included on each gel as an internal control. Estimates of relative uterine haptoglobin in the samples were obtained by recording blot images on computer files (Adobe Photoshop, San José, CA, USA) and scanning these files for relative density at the location of the standard haptoglobin band (day 6.75 pregnant rabbit uterine sample; PhospoImager®, ImageQuant®; Molecular Dynamics Inc., Sunnyvale, CA, USA). Under non-reducing conditions, the 42 kilodalton (kDa) β-haptoglobin is localized as part of a tetramer of α and β subunits with a MW of ~120 000 (Hoffman *et al.*, 1996). Volume densities were recorded and blots of individual treatment groups normalized to the density of the 120 kDa standard on each blot.

Glucocorticoid/anti-glucocorticoid assays

Young male rats (100–120 g) were adrenalectomized using aseptic surgical technique and treated s.c. with control vehicle, dexamethasone

(glucocorticoid reference standard), or CDB-2914 or mifepristone for 3 consecutive days (10/group). Twenty-four hours after the final dose, rats were killed, the final body weight recorded, and the thymus gland excised, trimmed, blotted, and weighed (Ringler *et al.*, 1964). For the anti-glucocorticoid bioassay, CDB-2914 or mifepristone were given orally in an attempt to block s.c. administered dexamethasone- or methylprednisolone-induced thymus involution.

Post-coital anti-fertility/anti-progestational activity in the mated rabbit

In order to determine whether CDB-2914 treatment exhibited post-coital anti-fertility activity during tubal egg transport, adult female rabbits (4–6/group) were treated with increasing daily doses of CDB-2914 from days 0–3 post-mating. The post-coital anti-fertility efficacy of a single oral dose of 16, 32 or 64 mg CDB-2914 was also assessed in female rabbits. The rabbits were killed on or about day 10 post-mating and the number and condition of implantation sites were determined. In other studies, female rabbits were treated orally with either the control vehicle (10% ethanol in sesame oil, $n = 7$) or with increasing doses of CDB-2914 ($n = 6$/group) on days 0–5 post-mating (day 0 = day of mating). Uteri were excised on day 6, the number of blastocysts was counted and flushed from the uterus with TBS-PI. A 2 cm uterine segment from each horn was fixed for histological evaluation (McPhail, 1934). Uterine incubation fluid samples were obtained and the relative amount of haptoglobin determined as described above.

Statistical analysis

Parametric analyses were performed on data that demonstrated homogeneity of variance and were normally distributed. Non-continuous data or continuous data that did not meet these criteria were analysed using non-parametric methods. SigmaStat® version 1.0 (Jandel Scientific, San Rafael, CA, USA) and SigmaPlot® version 2.0 (Jandel Scientific) were used for analyses. Significance was determined at an alpha of 0.05. A Pearson product moment correlation was performed to determine if there were significant associations among amounts of uterine haptoglobin, McPhail indices, uterine weight, and number of blastocysts.

Results

Progestational/anti-progestational assays

Reversal of CDB-2914 post-coital anti-fertility activity with progesterone in the rat

Recently, it was reported that a single oral dose of 2 mg CDB-2914 was highly effective in blocking pregnancy in the rat when given orally on either day 4 or 5 post-mating (Reel *et al.*, 1998). Since this effect was presumably due to antagonism of endogenous progesterone, it was reasoned that it should be possible to block the post-coital anti-fertility activity of CDB-2914 by the concurrent administration of progesterone. Subcutaneous injection of 10 mg progesterone/rat at 0730 and 1530 h along with 2 mg of CDB-2914 orally at 0930 h on day 4 post-mating resulted in the maintenance of pregnancy in seven of 10 rats (Table I). Confirming our earlier observation, the 2 mg dose of CDB-2914 alone abolished pregnancy in 10 of 10 rats. As expected, progesterone treatment alone had no adverse effects on pregnancy (Table I).

Stimulation and inhibition of endometrial glandular arborization in the immature female rabbit

Our data (not shown) confirm the anti-progestational activity of CDB-2914 as indicated by decreased endometrial glandular arborization and uterine weight in the immature rabbit bioassay (Cook *et al.*, 1992, 1994). CDB-2914 was three to four times more potent by the s.c. than the oral route [ED_{50} = 1.39 mg versus 5.19 mg (total doses), respectively]. CDB-2914 administered orally to oestradiol-primed immature female rabbits failed to stimulate endometrial glandular arborization or to increase uterine weight (data not shown) at oral doses that exerted anti-progestational activity, whereas the reference progestogen, levonorgestrel, resulted in a dose-dependent increase in both endpoints (data not shown).

Induction and antagonism of uterine haptoglobin synthesis and secretion in the immature female rabbit

Uterine haptoglobin was detected as a single immunoreactive 120 kDa band. Haptoglobin was barely detectable in the uterine incubation fluid of immature female rabbits treated with oestradiol alone, but was significantly increased ($P < 0.05$) in uterine incubation fluid obtained from oestrogen-primed immature rabbits subsequently treated with progesterone (data not shown). CDB-2914 significantly inhibited ($P < 0.05$) progesterone-induced uterine haptoglobin production and release in a dose-dependent manner (data not shown). Although no correlation ($P < 0.05$) was apparent between uterine weight and amount of haptoglobin in immature rabbits, a significant positive correlation ($P < 0.01$) existed between the production of uterine haptoglobin and the degree of endometrial glandular arborization.

Glucocorticoid/anti-glucocorticoid assays

Neither CDB-2914 nor mifepristone exhibited glucocorticoid activity in adrenalectomized male rats at total doses as high

Table I. Progesterone blockade of CDB-2914 post-coital anti-fertility activity in the mated rat

Treatment group[a]	No. pregnant/ No. mated	No. normal concepti per pregnant rat (mean ± SE)	No. resorbing implants per pregnant rat (mean ± SE)
Vehicle/vehicle	9/10	10 ± 1	0.4 ± 0.2
Progesterone/CDB-2914	7/10	9 ± 2	1.4 ± 0.5
Vehicle/CDB-2914	0/10[b]	-	-
Progesterone/vehicle	9/10	13 ± 1	0.2 ± 0.1

[a]Rats received either vehicle or CDB-2914 at 2 mg/rat orally on day 4 post-mating and either vehicle or progesterone at 10 mg/rat×2 s.c. on day 4 post-mating.
[b]Significantly different ($P < 0.05$) from the vehicle control group based on a z-test of proportions.

as 10 mg/rat (data not shown). In contrast, both compounds had detectable anti-glucocorticoid activity at a total dose of 9 mg/rat when dexamethasone (30 µg/rat total dose) was used as the reference glucocorticoid (data not shown). However, neither compound was able to block completely dexamethasone-induced thymus involution. Therefore, an additional anti-glucocorticoid assay was performed using the less potent reference glucocorticoid, methylprednisolone (Kupfer and Partridge, 1970). Both CDB-2914 and mifepristone exhibited a dose-dependent inhibition of methylprednisolone-induced thymus involution (Figure 1). At a total dose of 12 mg/rat, mifepristone and CDB-2914 partially prevented methylprednisolone-induced thymus involution, however, mifepristone was approximately twice as potent as CDB-2914 in this regard.

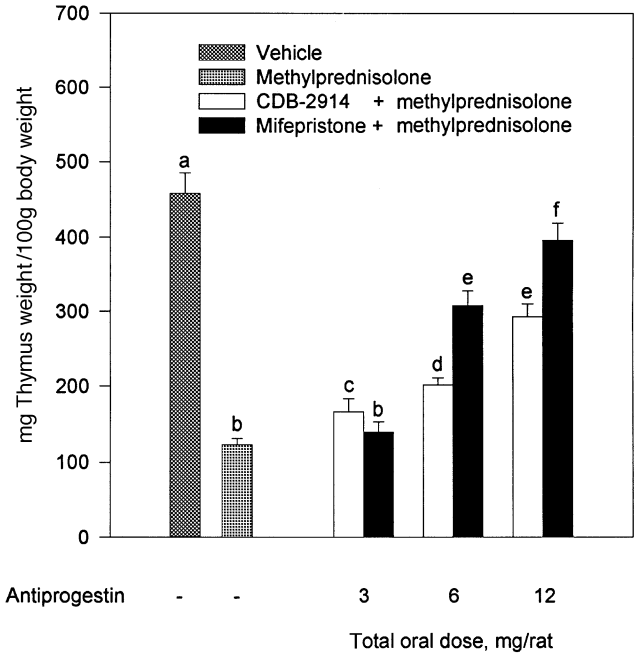

Figure 1. Anti-glucocorticoid activity of CDB-2914 and mifepristone in adrenalectomized male rats, as measured by thymus involution. Total s.c. dose of methylprednisolone was 1 mg/rat. Bars and brackets represent mean ± SE, $n = 10$/group. Bars with different letters were significantly different from each other based on a one-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls multiple range test to determine all pairwise differences.

Post-coital anti-fertility/anti-progestational activity in the mated rabbit

Oral administration of CDB-2914 on days 0–3 post-mating, during the peri-ovulatory and tubal egg transport period, resulted in a dose-dependent reduction in implantation sites and pregnancy rates (Table II). Pregnancy was blocked completely at an oral dose of 16 mg/rabbit/day over the 4 day treatment period.

In addition, CDB-2914 given orally on days 0–5 post-mating exhibited anti-progestational and post-coital anti-fertility activity in adult female rabbits (Table III). Dose-dependent inhibition of uterine weight and glandular arborization was observed in CDB-2914-treated mated rabbits and was accompanied by a decrease in pregnancy rate. At a daily dose of 8 mg/rabbit, CDB-2914 resulted in a significant reduction ($P < 0.05$) in the number of uterine blastocysts and at 16 mg/rabbit/day completely blocked pregnancy.

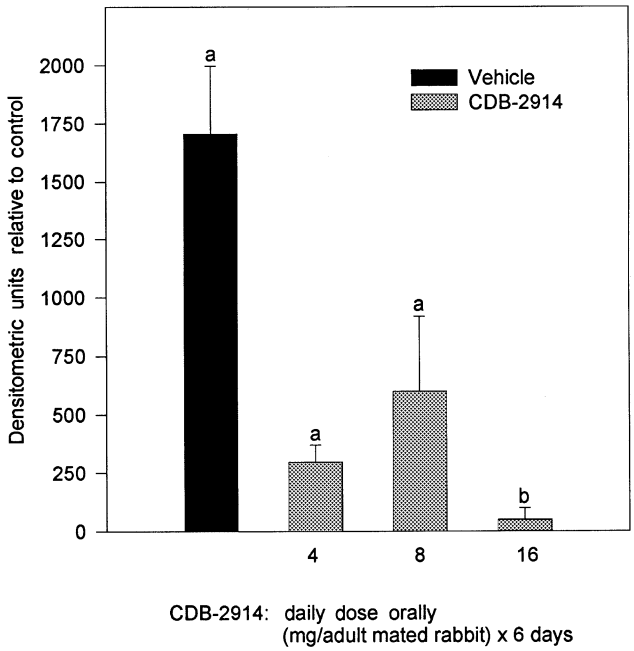

Figure 2. Relative amount of uterine haptoglobin synthesis and secretion in mated adult female rabbits (data from same rabbits as in Table III). Bars and brackets represent mean ± SE. Bars with different letters were significantly different ($P < 0.05$) from each other based on a Kruskal–Wallis ANOVA on ranks followed by Dunn's multiple range test.

Table II. Post-coital anti-fertility activity of CDB-2914 in the rabbit when administered orally from days 0–3 post-mating[a]

Treatment group[a]	No. pregnant/ No. ovulated	No. normal concepti per pregnant rabbit (mean ± SE)	No. resorbing implants per pregnant rabbit (mean ± SE)
Vehicle	4/4	9 ± 2	0.8 ± 0.8
2.0	5/5	8 ± 1	0.0 ± 0.0
4.0	4/6	6 ± 2	1.3 ± 0.5
8.0	2/6	4	0
16.0	0/6[b]	–	–

[a]Day 0 = day of mating.
[b]Significantly different ($P < 0.05$) from the vehicle control group based on a z-test of proportions.

CDB-2914 administered on days 0–5 post-mating also depressed the synthesis and secretion of uterine haptoglobin, a progesterone-induced protein implicated in the implantation process (Figure 2; Hoffman *et al.*, 1996; Olson *et al.*, 1997). Mated adult rabbits treated with vehicle alone had increased uterine haptoglobin present in the uterine incubation fluid (Figure 2) which coincided with the presence of blastocysts in the uteri (Table III). Significant positive correlations were observed among amount of uterine haptoglobin and uterine weight ($P < 0.01$), degree of endometrial glandular arborization ($P < 0.01$), and the number of blastocysts present in the uterus ($P < 0.01$).

In another study, a single oral dose of 16 mg CDB-2914/rabbit was ineffective at blocking pregnancy when administered on either day 0, 1, 2, or 3 post-mating (data not shown). Hence, in a subsequent assay, higher single oral doses of 32 and 64 mg CDB-2914/rabbit were administered orally on day 4, 5, or 6 post-mating (6/group). The two higher doses were given nearer the time of implantation in the rabbit based on the observation that CDB-2914 exhibited maximal effectiveness in inhibiting pregnancy in the rat on days 4 and 5 post-mating (Reel *et al.*, 1998). A single oral dose of 32 mg CDB-2914/rabbit tended to reduce the pregnancy rate when administered on days 5 and 6 post-mating, but did not inhibit pregnancy when given on day 4 post-mating (Table IV). In addition, rabbits that were found pregnant following dosing on day 5 or 6 post-mating tended to have a reduced number of normal concepti and a slight increase in resorbing implantation sites. A single oral dose of 64 mg CDB-2914/rabbit on day 4, 5, or 6 post-mating blocked pregnancy in all rabbits (Table IV).

Discussion

In the present study, CDB-2914 acted as a pure anti-progestin with no agonist activity in the immature rabbit bioassay. Anti-progestational activity was presumably mediated through competitive binding to PR since CDB-2914 demonstrated a relatively high binding affinity to PR (Cook *et al.*, 1992, 1994; unpublished data) and high doses of progesterone were able to inhibit the post-coital anti-fertility activity of CDB-2914 in the rat. Mifepristone has also been classified as a pure antagonist with no agonist properties based on data in the immature rabbit bioassay (Rauch *et al.*, 1985) and inhibition of endometrial glandular development in the uteri of rhesus monkeys (Koering *et al.*, 1986; Ghosh *et al.*, 1996) and women (Gravanis *et al*, 1985; Greene *et al*, 1992; Gemzell-Danielsson *et al.*, 1996, 1997). In a recent phase I clinical trial, CDB-2914 was reported to inhibit endometrial development in women in a dose-dependent manner (Passaro *et al.*, 1997). Although mifepristone demonstrated pure antagonist activity in the rabbit bioassay, progestational activity has been detected in the endometrium of rhesus monkeys or women treated with mifepristone in the absence of progesterone (Gravanis *et al*, 1985; Koering *et al.*, 1986; Wolf *et al.*, 1989; Hodgen *et al.*, 1994). Hence, the hormonal milieu appears to be important in determining progestational agonist/antagonist activity. In an in-vitro transcriptional assay system, both mifepristone and CDB-2914 demonstrated pure antagonist activity, whereas other 11β-aryl analogues were classified as mixed agonists/antagonists (Wagner *et al.*, 1996). These results for CDB-2914 were recently confirmed in the T47D cell alkaline phosphatase assay (Wagner *et al.*, 1999). Collectively, these data demonstrated that CDB-2914 acted as a pure progesterone antagonist in in-vivo and in-vitro systems.

In conjunction with these effects on the endometrium, anti-progestins inhibit pregnancy. CDB-2914 has been shown to prevent pregnancy in rats when administered at a low daily dose or as a post-coital contraceptive (Reel *et al.*, 1998). This post-coital anti-fertility activity is due to progesterone antagonism and is reversible as shown in the present study. CDB-2914 was also more potent than mifepristone in terminating early pregnancy in the rat following oral administration (Teutsch and Philibert, 1994), but was only equally effective as an abortifacient in monkeys (Tarantal *et al.*, 1996). In the present study, CDB-2914 was able to completely block pregnancy in rabbits when administered orally after mating at doses as low as 16 mg/rabbit/day for 5 days or at a single high dose (64 mg/rabbit). These anti-fertility effects of CDB-2914 are probably mediated through multiple mechanisms involving progesterone action. Mifepristone exhibited contragestational activity when administered orally to rabbits at a daily dose of 5 mg/kg (17.5–20 mg/rabbit) during days 8, 9 and 10 post-mating (Chang *et al.*, 1993). Mifepristone partially blocked ovulation in the rabbit at a single s.c. dose of 150 mg (17% ovulation rate, Kanayama *et al.*, 1996) or at daily s.c. doses of 10 mg/kg for 3 days

Table III. Post-coital anti-fertility and anti-progestational activity of CDB-2914 in the rabbit when administered orally from days 0–5 post-mating[a]

Treatment group[a] (mg/rabbit/day × 6 days)	Uterine weight (g, mean ± SE)	McPhail index (mean ± SE)	No. pregnant/ No. ovulated	No. blastocysts/pregnant rabbit (mean ± SE)
Vehicle	12.22 ± 1.18[b]	4.0 ± 0.0[b]	6/7	11 ± 1[b]
4	7.11 ± 0.83[c]	2.8 ± 0.5[b,c]	4/6	11 ± 1[b]
8	7.43 ± 1.47[c]	1.7 ± 0.5[c]	4/6	6 ± 2[c]
16	4.10 ± 0.31[c]	0.0 ± 0.0[c]	0/6[d]	–

[a]Day 0 = day of mating.
[b,c]Means with different superscript letters were significantly different ($P < 0.05$) from each other based on a one-way analysis of variance followed by Student–Neumann–Keuls multiple range test to determine all pairwise differences.
[d]Significantly different ($P < 0.05$) from the vehicle control group based on a z-test of proportions.

Table IV. Post-coital anti-fertility activity of a single 32 or 64 mg-dose of CDB-2914 in the rabbit when administered orally on day 4, 5, or 6 post-mating[a]

Treatment group implants (mg/rabbit/day)	No. pregnant/ No. ovulated	No. normal concepti per pregnant rabbit (mean ± SE)	No. resorbing implants per pregnant rabbit (mean ± SE)
Vehicle[b]	6/6	9 ± 1	0.2 ± 0.2
Day 4			
32 mg	6/6[c]	6 ± 2	0.5 ± 0.3
64 mg	0/6	–	–
Day 5			
32 mg	4/6	8 ± 1	1.5 ± 0.9
64 mg	0/6	–	–
Day 6			
32 mg	3/6[d]	3 ± 2	1.0 ± 0.6
64 mg	0/6	–	–

[a]Day 0 = day of mating.
[b]Rabbits received a single dose of vehicle on day 6 post-mating.
[c]Two pregnant rabbits had only resorbing implants.
[d]One pregnant rabbit had only resorbing implants.

(52% ovulation rate, Chen *et al.*, 1995). CDB-2914 was also capable of completely blocking ovulation in the rat when administered at a single oral dose of 2 mg during pro-oestrus (Reel *et al.*, 1998). Mifepristone has also been shown to inhibit endometrial secretory production of uteroglobin in the rabbit at doses of 10 mg/day for 6 days (Rauch *et al.*, 1985). In the present study, the anti-fertility effect of CDB-2914 in rabbits was accompanied by a decrease in uterine haptoglobin. Uterine haptoglobin is a progesterone-dependent protein produced by the luminal epithelial cells during early pregnancy in the rabbit (Hoffman *et al.*, 1996; Olson *et al.*, 1997). Amounts peak during the time of blastocyst attachment and implantation (days 6–7 post-mating). Since this protein is implicated in the implantation process, CDB-2914 inhibition of haptoglobin production, or other secretory products, may be one of the mechanisms by which CDB 2914 blocks pregnancy. Both mifepristone and CDB-2914 have been shown to retard endometrial development in women (Gemzell-Danielsson *et al.*, 1994; Passaro *et al.*, 1997). The effects of mifepristone on the primate endometrium included inhibition of both glandular secretory development and stromal growth and oedema (Gemzell-Danielsson *et al.*, 1994; Greb *et al.*, 1999). Administration of single 10 mg dose of mifepristone to women 5 days following unprotected intercourse reduced pregnancy rates (1.2%) indicating the effectiveness of anti-progestins as emergency contraceptives (Piaggio *et al.*, 1999). Collectively, these data support the development of anti-progestins for contraceptive purposes and demonstrate their ability to block pregnancy through multiple mechanisms.

Like mifepristone, CDB-2914 demonstrated in-vitro binding to the glucocorticoid receptor (GR), however, the receptor binding affinity (RBA) for CDB-2914 to GR tended to be lower than mifepristone (Wagner *et al.*, 1999; unpublished data). Both CDB-2914 and mifepristone demonstrated anti-glucocorticoid activity with no agonist activity in the rat thymolytic bioassay. These data support previously published studies indicating that CDB-2914 and mifepristone bind the GR with high affinity and act as glucocorticoid antagonists (Gagne *et al.*, 1985, 1986; Busso *et al.*, 1987; Cook *et al.*, 1992, 1994; Wagner *et al.*, 1999). Mifepristone was a more potent anti-glucocorticoid than CDB-2914 in the rat thymolytic bioassay supporting qualitatively the in-vitro findings in a glucocorticoid reporter gene assay in which mifepristone was found to be 100-fold more potent than CDB-2914 at inhibiting dexamethasone-stimulated transcriptional activity (Wagner *et al.*, 1999). However, the discrepancy between the relative potency of mifepristone compared to CDB-2914 in the in-vivo thymolytic bioassay (two-fold) versus the in-vitro transcriptional assay (100-fold) indicates that the in-vitro system does not quantitatively reflect the in-vivo situation. *In vivo*, the N-mono- and didemethylated and hydroxylated metabolites of mifepristone may contribute as much as 47–69% of the anti-glucocorticoid activity based on their RBA to GR (Heikinheimo *et al.*, 1987). Although the putative N-mono- and didemethylated and hydroxylated metabolites of CDB-2914 demonstrated binding to GR (unpublished data), the binding affinities were lower than dexamethasone, whereas the mifepristone metabolites demonstrated greater binding affinity to GR than dexamethasone (Heikinheimo *et al.*, 1987). This finding suggests that the putative CDB-2914 metabolites contribute less anti-glucocorticoid activity *in vivo* than do mifepristone's metabolites.

Although mifepristone and CDB-2914 were equipotent when delivered directly to the uterine lumen, CDB-2914 was more potent than mifepristone when administered orally (Cook *et al.*, 1994). This finding does not correspond directly to the ability of mifepristone and CDB-2914 to bind PR *in vitro* and may reflect differences in the bioavailability of the drug or the relative potency of the proximal metabolites following oral administration. The putative N-monodemethylated metabolite of CDB-2914 demonstrated oral anti-progestational and anti-fertility activity in immature female rabbits and mated female rats respectively (unpublished data) indicating that this proposed metabolite may contribute to the overall anti-progestational activity of CDB-2914. The pharmacokinetic profile of CDB-2914 in rhesus monkeys indicated that CDB-

2914 had 4.7 or 5.3 times greater oral bioavailability than mifepristone when administered as an oral aqueous suspension or in gelatin capsules respectively (Larner *et al.*, 1996). Mifepristone serum concentrations peaked earlier in monkeys than CDB-2914, but had a shorter elimination half-life than CDB-2914. These data indicated that CDB-2914 had greater oral bioavailability than mifepristone which may account for its greater oral anti-progestational potency.

In conclusion, the data from these studies demonstrate that CDB-2914 is a potent orally active progesterone antagonist with weak anti-glucocorticoid activity. CDB-2914 displayed post-coital anti-fertility activity in the mated rabbit which could be reversed by progesterone treatment in the mated rat. Finally, since CDB-2914 possessed greater oral anti-progestational potency and less anti-glucocorticoid activity than mifepristone, it may be a more specific anti-progestin in clinical use.

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