Circulating concentrations of the antiprogestins CDB-2914 and mifepristone in the female rhesus monkey following various routes of administration*

J.M. Larner1,3, J.R. Reel1,4 and R.P. Blye2

1BIOQUAL, Inc., Rockville, MD, 20850, USA and 2Contraception and Reproductive Health Branch, National Institute of Child Health and Human Development, Rockville, MD, 20892, USA
3Present address: Alexion Pharmaceuticals, Inc., New Haven, CT 06511, USA
4To whom correspondence should be addressed

The overall aim of these studies was to investigate the oral and i.m. bioavailability of CDB-2914 in intact female rhesus monkeys, and to compare the serum concentrations of CDB-2914 with that of mifepristone following oral administration. In the first study, a 50 mg bolus of CDB-2914 per monkey was administered intravenously, orally or intramuscularly. The area under the serum concentration–time curve 120 h (AUC0–120 h) following i.v. injection was 33320 ± 2718 ng/ml•h, and that for oral administration was 10,464 ± 3248 ng/ml•h. Thus, the oral bioavailability of CDB-2914 equivalents was 56%. The AUC0–168 h following i.m. injection was 11,226 ± 1130 ng/ml•h. Therefore, the i.m. bioavailability of CDB-2914 equivalents was 62%. In the second study, the serum concentrations of CDB-2914 and mifepristone equivalents were compared following an oral bolus dose in two different formulations. When administered at 5 mg/kg in aqueous suspending vehicle (ASV), the mean peak serum concentration (Cmax) of CDB-2914 equivalents (192 ± 64 ng/ml) occurred at 5 ± 1 h, whereas the Cmax of mifepristone equivalents (82 ± 25 ng/ml) occurred at 3 ± 1 h. Following administration in gelatin capsules (35 mg/mo), the Cmax of CDB-2914 equivalents (129 ± 24 ng/ml) occurred at 5 ± 1 h, while the Cmax of mifepristone equivalents (31 ± 8 ng/ml) occurred at 3 ± 1 h. The serum concentration (AUC0–120 h) of CDB-2914 equivalents was 4.7- or 5.3-fold greater than that of mifepristone equivalents when administered orally in ASV or gelatin capsules respectively. The serum protein binding characteristics of CDB-2914 were also studied. CDB-2914 bound to human α1-acid glycoprotein (AAG), but not with as high an affinity as mifepristone. In contrast, neither CDB-2914 nor mifepristone bound with high affinity to AAG, corticosteroid binding globulin or sex hormone binding globulin in monkey serum. Collectively, these results indicated that CDB-2914 was more efficiently absorbed than mifepristone following oral administration to female rhesus monkeys.

Key words: antiprogestin/bioavailability/contraception/protein binding/rhesus monkey

Introduction

Antiprogestins, as exemplified by mifepristone, have broad therapeutic potential in the treatment of conditions such as cervical ripening, induction of labour, endometriosis and uterine fibroids, as well as use as contraceptive agents (Spitz and Bardin, 1993). The antiprogestin CDB-2914 (17α-acetoxy-11β-(4-N,N-dimethylanilinophenyl)-19-norpregna-4,9-diene-3,20-dione) is structurally related to mifepristone, but possesses 17β-acetyl and 17α-acetoxy groups instead of 17β-hydroxy and 17α-propynyl groups (Figure 1). Thus, CDB-2914 may be considered a derivative of 19-norpregesterone, whereas mifepristone is derived from 19-nortestosterone. CDB-2914 is also known variously as RTI 3021-12 (Cook et al., 1994), RU 44675 (Teutsch and Philibert, 1994) and HRP 2000 (Tarantal et al., 1996). CDB-2914, like mifepristone and other 11β-aryl substituted antiprogestins, binds to progesterone and glucocorticoid receptors (PR, GR) with high affinity (Cook et al., 1994; Hild-Petito et al., 1996). In the rat, CDB-2914 had an oral anti-ovulatory potency 8-fold greater than that of mifepristone (Reel et al., 1998). Further evaluation in classical antiprogestational bioassays in the rabbit indicated that CDB-2914 was about equipotent to mifepristone when administered subcutaneously, but two to three times more potent than mifepristone when administered orally (Cook et al., 1994). Thus, it appeared that differences in serum concentrations between CDB-2914 and mifepristone following oral administration might account for the differences in oral biological potencies.

The pharmacokinetics of mifepristone, the most widely characterized 11β-aryl substituted antiprogestin, have been studied mostly in women following oral administration, although some studies have been conducted in monkeys and rats (Deraedt et al., 1985; Lähteenmäki et al., 1987; Heikinheimo et al., 1994). Following oral administration in humans, mifepristone is characterized by rapid absorption, with peak serum concentrations occurring in 1–2 h and a long half-life of 25–30 h (Heikinheimo, 1997). The serum profiles of three of the known metabolites of mifepristone, the monodemethylated and hydroxylated forms, show the same pattern of disappearance from the circulation as the parent steroid. In addition, the monodemethylated and hydroxylated metabolites bind to human uterine PR and placental GR (Heikinheimo et al., 1987a). In the cynomolgus monkey and

*Presented in part at the 10th International Congress of Endocrinology, San Francisco, California, 1996, Abstract P2-691

© European Society of Human Reproduction and Embryology
the rat, absorption was also rapid; however, in contrast to humans, the elimination half-lives were 15 h and 1–2 h respectively (Deraedt et al., 1985; Heikinheimo et al., 1994). The most likely explanation for the differences among the three species appears to be the lack of a high-affinity mifepristone binding protein in monkey and rat sera (Moguilewsky and Philibert, 1985; Heikinheimo et al., 1994). In humans, α1-acid glycoprotein (AAG) has been identified as the high-affinity binding protein for mifepristone (Grimaldi et al., 1992). In addition, several studies have shown a significant correlation between serum mifepristone and AAG concentrations (Heikinheimo et al., 1987b; Grimaldi et al., 1992). Serum protein binding is also known to influence the pharmacokinetics of steroid hormones such as cortisol, and contraceptive steroids such as levonorgestrel and gestodene (Westphal, 1986; Juchem and Pollow, 1990; Fotherby, 1995).

The present study was undertaken to characterize the circulating concentrations of CDB-2914 equivalents in female rhesus monkeys following a single 50 mg dose of CDB-2914 administered in aqueous or oily formulations by the i.v., oral or i.m. routes, and to compare the serum concentrations of CDB-2914 and mifepristone given orally in aqueous suspending vehicle (ASV) or gelatin capsules. In conjunction with the kinetic studies, the binding of CDB-2914 and mifepristone by purified human AAG and by human and rhesus monkey corticosteroid binding globulin (CBG) and sex hormone binding globulin (SHBG) was also investigated. Furthermore, the high-affinity binding of these two anti-progestins by proteins in diluted rhesus monkey serum was studied in an attempt to determine whether this phenomenon influenced circulating concentrations.

**Materials and methods**

**Steroids and vehicles**

The chemical structures for CDB-2914, mifepristone and progesterone are shown in Figure 1. CDB-2914 (17α-acetoxy-[11β-(4-N,N-dimethylaminophenyl)]-19-norpregna-4,9-diene-3,20-dione), the 3-carboxymethyloxime–bovine serum albumin (BSA) conjugate of CDB-2914 and the 3-carboxymethyloxime–histamine conjugates of both CDB-2914 and mifepristone (11β-[4-N,N-dimethylaminophenyl],17β-hydroxy,17α-propynyl estra-4,9-dien-3-one) were synthesized by the Southwest Foundation for Biomedical Research (San Antonio, TX, USA) under contract N01-HD-1-3137 to the Contraception and Reproductive Health Branch, National Institute of Child Health and Human Development, Rockville, MD, USA (NICHD). The monoclonal antibody against mifepristone was a gift from Dr Fortune Kohen (Weizman Institute of Science, Rehovot, Israel). The mono- and didemethylated and hydroxylated metabolites of mifepristone were gifts from Dr Pekka Lahenemaki (University of Helsinki, Helsinki, Finland). [N-C3H3]CDB-2914 ([3H]CDB-2914; specific activity, 80 Ci/mmol), [N-C3H3]mifepristone ([3H]mifepristone; specific activity, 80 Ci/mmol), and unlabelled mifepristone were provided by the Contraception and Reproductive Health Branch, NICHD. [3H]CDB-2914 and [3H]mifepristone were purified before use by high-performance liquid chromatography (HPLC) using a LiChrosorb DIOL column (4.6×250 mm; EM Science Div., EM Industries, Gibbstown, NJ, USA) with methylene chloride:iso-octane (1:1) as the mobile phase and a flow rate of 1 ml/min. [1125]Na (pH 8–11), [1,2-3H]cortisol (52 Ci/mmol) and [6,7-3H]5α-dihydrotestosterone (DHT; 43.7 Ci/mmol) were obtained from NEN-Dupont (Boston, MA, USA). Purified human AAG was purchased from Sigma (St Louis, MO, USA). All other chemicals were analytical grade. Solutions of CDB-2914 were formulated in ethanol:water (7:3) for i.v. and oral administration, and in ethanol:sesame oil (1:4) for i.m. administration. Aqueous suspensions of CDB-2914 and

![Figure 1. Chemical structures of CDB-2914, mifepristone and progesterone.](image-url)
mifepristone were prepared in aqueous suspending vehicle [ASV; 0.9% NaCl (w/v), 0.5% carboxymethyl cellulose (w/v), 0.4% (v/v) polysorbate 80 and 0.9% (v/v) benzyl alcohol in distilled water]. Gelatin capsules containing CDB-2914 or mifepristone and Avicel® excipient were formulated by the National Institute of Health (NIH) Clinical Center Pharmacy (NIH, Bethesda, MD, USA).

$[^{125}]$CDB-2914 and $[^{125}]$mifepristone were prepared by iodination of the respective 3-carboxymethylloxime–histamine conjugates using a modified chloramine-T method (Niswender et al., 1975) and purified twice by HPLC using a 5 μm LiChrosorb RP-18 column (4.6×250 mm; Merck, Darmstadt, Germany) and a gradient of acetonitrile:water (40:60) to 100% acetonitrile over 40 min at a flow rate of 1 ml/min.

**Animals**

Nine intact, regular cycling, female rhesus monkeys (*Macaca mulatta*) of mean (± SE) bodyweight 7.6 ± 0.5 kg were used in these studies. Menstrual cycles were monitored daily by vaginal swabs to detect the presence and quantity of blood. Male New Zealand White rabbits weighing 3–4 kg were employed for antisera production. Animals were maintained in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and the environmental conditions of the animal rooms were maintained as recommended by the National Research Council (National Research Council, 1996) to the maximum extent possible. The Institutional Animal Care and Use Committee of BIOQUAL approved all study protocols.

The same three to four monkeys were used for both CDB-2914 and mifepristone pharmacokinetic studies. The monkeys were not anaesthetized during dosing or blood collection. The animals were held in a chair restraint during oral and i.v. dosing, and for an additional hour after dosing. In the i.v. study, monkeys were injected at random times in their menstrual cycles. In all other studies, the monkeys were dosed at midcycle (days 13–16; day 1 = first day of menses) and fasted overnight before dosing and for 4 h after oral dosing, which was performed via a gastric catheter. When test compounds were administered as aqueous suspensions the catheter was flushed with additional vehicle to ensure complete delivery. Gelatin capsules containing test compounds were forced through the catheter using a bolus of air. Blood samples were collected by venepuncture from the cephalic, saphenous or femoral veins immediately before and at various times after dosing. Serum was obtained by centrifugation at 2600 × g for 15 min, and stored at –20°C until assayed for CDB-2914 or mifepristone and their immunoreactive metabolites.

**Antisera**

Polyclonal antisera against CDB-2914 3-carboxymethylloxime–BSA were raised in four male rabbits using a previously described method (Vaitukaitis et al., 1971). Initially, each rabbit was immunized with divided intradermal injections of 4 mg of the immunogen emulsified in Freund’s complete adjuvant:saline (1:1, v/v). This was followed by booster immunizations in Freund’s incomplete adjuvant:saline (1:1, v/v) at 4-week intervals until no further increases in antibody titres were obtained. Sera from bleed nos. 3–5 from the rabbit (#67192) with the highest antibody titre and the greatest CDB-2914 specificity were pooled and used for assay development and validation. This antisera was titrated with each new preparation of $[^{125}]$CDB-2914 to determine the final dilution that resulted in 30–40% specifically bound radioligand. The N-mono- and $\text{-didemethylated putative metabolites of CDB-2914 exhibited 76% and 59% cross-reactivity with the antisera respectively (Table I). Progesterone, mifepristone, oestradiol, oestrone, cortisol, testosterone and the putative 17α-

<table>
<thead>
<tr>
<th>Table I. Cross-reactivities (%) of putative CDB-2914 metabolites and known mifepristone metabolites with their respective antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolite</td>
</tr>
<tr>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Monodemethyl</td>
</tr>
<tr>
<td>Didemethyl</td>
</tr>
<tr>
<td>Hydroxy</td>
</tr>
</tbody>
</table>

hydroxylated metabolite of CDB-2914 exhibited <1% cross-reactivity.

**Radioimmunoassay**

Radioimmunoassay data were analysed using a four-parameter sigmoidal computer program (RiaSmart™ Data Reduction Program, Packard Instrument Co., Meriden, CT, USA). Quality control parameters including percentage specific bound (B0), percentage non-specific bound (NSB), EC50, EC60 and EC80 intercepts, slope and correlation coefficient were calculated routinely by the program for all standard curves. The usable range of the CDB-2914 standard curve was 1 to 400 pg/tube, with a mean (± SE) mid-point of 30 ± 2 pg/tube (n = 10) and a mean slope of −0.91 ± 0.03 (n = 10). The average limit of detection was 1 ± 0.1 pg/tube. The recovery of CDB-2914 from low-, mid- and high-concentration quality control serum pools run with each assay averaged 100 ± 8% (n = 3). The inter- and intra-assay coefficients of variation were 7% and 7% for CDB-2914, and 9% and 18% for mifepristone respectively.

Serum samples from five monkeys dosed orally with 5 mg CDB-2914/kg were assayed for CDB-2914 and its putative immunoreactive metabolites over a series of dilutions. Using a semi-log plot of percent bound versus concentration, the diluted serum samples decreased in a linear and parallel fashion to the CDB-2914 standard curve. Parallelism was assessed by comparison of the mean slopes calculated by RiaSmart™ for the regression lines (Altman, 1991). The mean slope of the standard curves (n = 6) was −0.86 [95% confidence intervals (CI) −0.76 to −0.96], and the mean slope of the diluted serum samples (n = 5) was −0.80 (95% CI −0.66 to −0.94).

The study serum samples were assayed for CDB-2914 or mifepristone equivalents as follows. CDB-2914 and mifepristone equivalents were extracted from sera with absolute methanol (1:4, v/v). Following centrifugation to remove the protein precipitate, the supernatant extracts were diluted to 20% methanol with assay buffer (50 mmol/l phosphate, pH 7.2, containing 0.1% gelatin), and then diluted further as required with assay buffer containing 20% methanol. Duplicate 200 μl aliquots were incubated overnight with 30 000 counts per min (c.p.m.) $[^{125}]$CDB-2914 or $[^{125}]$mifepristone and the respective antibody at 2–6°C. The final methanol concentration in the incubation mixture was 5%. Bound and free radioligand were separated using a dextran-coated charcoal method (Reel et al., 1979). Following precipitation of the charcoal by centrifugation (2600 g, 15 min), the supernatants were decanted and counted for 2 min each in a Packard Cobra™ II gamma counter. The serum equivalents for CDB-2914 or mifepristone were derived by correcting for aliquot volume and serum dilution factor. Two of the early metabolites of mifepristone in women, the N-mono- and $\text{-didemethylated products (Deraedt et al., 1985; Heikinheimo et al., 1987a), cross-reacted with the antibody at 86% and 57% respectively (Table I). It seems likely that these two metabolites were also produced in female monkeys, though no attempt was made at their isolation and identification. The serum concentrations were expressed as ng equivalents of CDB-2914 and mifepristone per ml in order to reflect the unknown contribution of
their proximal metabolites which have been found to cross-react with the antisera used.

**Serum protein binding**

The relative binding affinities (RBA) of SHBG and CBG for CDB-2914, mifepristone, progesterone, oestradiol (SHBG only) and dexamethasone (CBG only) were determined using a published method (Hammond and Lähteenmäki, 1983). Briefly, aliquots of pooled human serum and pooled female rhesus monkey serum were charcoal-stripped and incubated with 5 nmol/l $[^3H]5\alpha$-dihydrotestosterone (5$\alpha$-DHT) or 5 nmol/l $[^3H]$cortisol and reference standard or competitor concentrations from 1 to 800 nmol/l. Non-specific binding was measured in the presence of a 200-fold molar excess of unlabelled 5$\alpha$-DHT or cortisol in the SHBG and CBG assays respectively. The SHBG assay also contained 120 nmol/l cortisol to block binding of the competitors to serum CBG. Samples were incubated for 2 h at 4°C. Bound and free radioligands were separated using a dextrancoated charcoal method (Reel et al., 1979). Following precipitation of the charcoal by centrifugation at 2000 g for 20 min, the supernatants were decanted and counted. The EC$_{50}$ values for the 5$\alpha$-DHT and cortisol standard curves and the competitor curves were calculated using RiaSmart™. The RBA (%) of the competitors were calculated using the following equation: (EC$_{50}$ of standard $\div$ EC$_{50}$ of competitor)$\times$100. Equilibrium dialysis was used to assess the binding by other serum proteins. Aliquots of female monkey serum diluted 1:100 in phosphate-buffered saline (PBS) (10 mmol/l NaHPO$_4$, 144 mmol/l NaCl, pH 7.2) containing 5% ethanol (PBS/5% ethanol) and 12.2 µmol/l purified human AAG (in PBS/5% ethanol) were dialysed against 5 nmol/l $[^3H]$CDB-2914 or 5 nmol/l $[^3H]$mifepristone. Incubations were carried out at 37°C, and both dialysis chambers were sampled over time until equilibrium was reached. At equilibrium (8 h), the percent bound radioligand was calculated using the following equation: ($[^3H]$ c.p.m. in serum protein chamber) – ($[^3H]$ c.p.m. in radioligand chamber) / ($[^3H]$ c.p.m. in serum protein chamber)$\times$100.

**Pharmacokinetic parameters and statistical analysis**

Parameters were estimated using the National Institutes of Health PROPHET Public Procedures drug modelling program (Holford, 1990). The PROPHET ‘drugmodel’ procedure was used to determine the mean peak serum concentration (C$_{\text{max}}$, ng/ml) and time to C$_{\text{max}}$ (h) values. The area under the curve (AUC) values (ng/ml h) were calculated using the trapezoidal rule. Statistical comparisons of C$_{\text{max}}$, time to C$_{\text{max}}$, and AUC were performed using Student’s t-test (SigmaStat, version 2.0; Jandel Scientific, San Raphael, CA, USA).

**Results**

**Circulating concentrations of CDB-2914 equivalents after i.v., oral and i.m. administration**

The time course of circulating concentrations of CDB-2914 equivalents varied depending on the route of administration. As expected, serum concentrations of CDB-2914 equivalents were highest immediately after a 50 mg bolus i.v. injection in 70% ethanol/bacteriostatic water and declined exponentially thereafter over the 72 h sampling period (Figure 2A).

In contrast, oral administration of 50 mg of CDB-2914 in the same vehicle resulted in gradual absorption from the gastrointestinal tract. CDB-2914 equivalents reached C$_{\text{max}}$ (239 to 761 ng/ml) at between 4 and 8 h after dosing, and decreased steadily thereafter to 72 h (Figure 2B). The mean C$_{\text{max}}$ for three monkeys following oral administration of 50 mg of CDB-2914 was 516 ng/ml, and the mean time to C$_{\text{max}}$ was 5 h (Table II).

As anticipated, gradual absorption was also observed following i.m. injection of 50 mg CDB-2914 in 20% ethanol/ sesame oil, with C$_{\text{max}}$ values reaching 156–328 ng/ml at between 8 and 24 h after administration (Figure 2C). The mean C$_{\text{max}}$ for three monkeys following i.m. injection of 50 mg CDB-2914 was 234 ng/ml, and the mean time to C$_{\text{max}}$ was 13 h (Table II).

In order to make estimates of bioavailability, the mean AUC were calculated for circulating concentrations of CDB-2914 equivalents following i.v. and i.m. administration (Table II). The ratio of the oral AUC$_{0-72\ h}$ to the i.v. AUC$_{0-72\ h}$ gave an oral bioavailability of 56% (Table II). In comparison, the ratio of the i.m. AUC$_{0-168\ h}$ to the i.v. AUC$_{0-72\ h}$ yielded an i.m. bioavailability of 62% (Table II).

**Circulating concentrations of CDB-2914 and mifepristone equivalents after oral administration in aqueous suspending vehicle (ASV) or gelatin capsules**

The oral pharmacokinetics of mifepristone have been studied extensively in women. Therefore, the results of a comparative study of CDB-2914 and mifepristone in female rhesus monkeys...
might provide some insight into what doses of CDB-2914 would be appropriate for phase I clinical trials pursuant to the treatment of endometriosis and uterine fibroids (Passaro et al., 1997). Oral administration of 5 mg CDB-2914/kg in ASV resulted in a mean $C_{\text{max}}$ of 192 mg/ml, and a mean time to $C_{\text{max}}$ of 5 h, whereas 5 mg mifepristone/kg in ASV gave a serum $C_{\text{max}}$ of 82 ng/ml and a time to $C_{\text{max}}$ of 3 h (Figure 3A, Table III). Although $C_{\text{max}}$ and time to $C_{\text{max}}$ were not significantly different between the two groups, the AUC$_{0-120h}$ for CDB-2914 was significantly greater ($P < 0.05$) than that for mifepristone. Hence, the AUC$_{0-120h}$ Ratio of CDB-2914 was 4.7-fold greater than that of mifepristone (Table III).

Oral administration of 35 mg CDB-2914 in a gelatin capsule yielded a mean $C_{\text{max}}$ of 129 ng/ml, and a time to $C_{\text{max}}$ of 5 h, whereas 35 mg of mifepristone in a gelatin capsule gave a serum $C_{\text{max}}$ of 31 ng/ml and a time to $C_{\text{max}}$ of 3 h (Figure 3B, Table III). In this instance, $C_{\text{max}}$ and time to $C_{\text{max}}$ were significantly greater ($P < 0.05$) for CDB-2914 than for mifepristone. As observed for the ASV formulation, the AUC$_{0-120h}$ was significantly greater ($P < 0.05$) for CDB-2914 than mifepristone following oral administration in a gelatin capsule. Indeed, the ratio of the CDB-2914 AUC$_{0-120h}$ to the mifepristone AUC$_{0-120h}$ was 5.3 (Table III).

### Serum protein binding

Neither CDB-2914 nor mifepristone bound to monkey or human SHBG or CBG. The protein binding assays for each species were performed on two separate occasions. The RBA values of SHBG for oestradiol, which served as a positive control, were 10 ± 1% and 3 ± 1% (mean ± SD, $n = 2$) for monkey and human respectively. The negative control, progesterone, did not compete with $[^{3}\text{H}]\alpha$-DHT for binding to either the monkey or human SHBG. For CBG, the mean RBA values for progesterone, the positive control, were 40 ± 6% and 10 ± 3% (mean ± SD, $n = 2$), for monkey and human respectively. As expected, dexamethasone did not compete with $[^{3}\text{H}]$cortisol for binding to CBG from either species.

By equilibrium dialysis, neither $[^{3}\text{H}]$CDB-2914 nor $[^{3}\text{H}]$mifepristone bound to serum proteins in 100-fold diluted monkey serum. The positive control, purified human AAG, bound 53 ± 10% (mean ± SD, $n = 4$) and 89 ± 5% (mean ± SD, $n = 2$) of the added $[^{3}\text{H}]$CDB-2914 and $[^{3}\text{H}]$mifepristone respectively. In addition, there was no evidence of specific binding of either antiprogestin by charcoal-stripped 75-fold diluted monkey serum using the same dextran-coated charcoal method as was employed to assess binding by SHBG and CBG.

### Discussion

This study is the first to determine the circulating concentrations and bioavailability of the antiprogestin, CDB-2914. Interes-
Circulating concentrations of CDB-2914 and mifepristone

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cmax (ng/ml)</th>
<th>Time to Cmax (b)</th>
<th>AUC0-120h (ng/ml·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDB-2914</td>
<td>192 ± 64</td>
<td>5 ± 1</td>
<td>4454 ± 1437**</td>
</tr>
<tr>
<td>Mifepristone</td>
<td>82 ± 25</td>
<td>3 ± 1</td>
<td>941 ± 170</td>
</tr>
<tr>
<td>AUC ratio</td>
<td>24**</td>
<td>1*</td>
<td>4.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gelatin capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (mg/kg)</td>
</tr>
<tr>
<td>129 ± 24*</td>
</tr>
<tr>
<td>Time to Cmax (b)</td>
</tr>
<tr>
<td>5 ± 1**</td>
</tr>
<tr>
<td>AUC0-120h (mg/monkey)</td>
</tr>
<tr>
<td>3476 ± 64*</td>
</tr>
<tr>
<td>AUC ratio</td>
</tr>
<tr>
<td>5.3</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n = 3–4).
AUC0-120h = AUC0-120h CDB-2914/AUC0-120h mifepristone. As CDB-2914 and/or its immunoreactive metabolites were still detectable in serum at 72 h (in study no. 1) following i.v. and oral administration, the AUC interval was extended to 0–120 h in this study involving oral administration of CDB-2914 and mifepristone.

**Significantly different (P < 0.05) from mifepristone (Student’s t-test).

*****Significantly different (P < 0.05) from mifepristone (Student’s t-test using log10 data transformation to achieve normally distributed values and homogeneity of variance).

CDB-2914 and mifepristone indicated that, due to the formulation and the route of administration, oral dosing formulation. The persistence of detectable concentrations of CDB-2914 in ASV, as indicated by the time to Cmax, was characterized by slow absorption and prolonged elimination. The relatively high bioavailability of CDB-2914 was accompanied by the persistence of detectable concentrations of CDB-2914 and/or its immunoreactive metabolites, and this may reflect enterohepatic recirculation as well as the progressive production and recirculation of proximal CDB-2914 metabolites. In-vivo studies in the human and rat have demonstrated that enterohepatic recirculation of mifepristone and its metabolites occurs following oral administration (Heikinheimo et al., 1989, 1994).

The persistence of detectable concentrations of CDB-2914 and its immunoreactive metabolites in serum following oral administration of CDB-2914 in ASV or gelatin capsules, or by i.m. injection, suggests significant extravasation of CDB-2914 and its putative immunoreactive metabolites. It is also possible that the profile of metabolites is different following oral administration as compared with i.m. injection. Early studies on the pharmacokinetics and metabolism of mifepristone indicated that, due to first-pass metabolism, the volume of distribution in humans was only 10% of body weight, whereas in rats and cynomolgus monkeys the volume of distribution represented 135% and 200% respectively of body weight (Deraedt et al., 1985). Of note was the finding that if the elimination half-life for mifepristone and its immunoreactive

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ASV</th>
<th>Gelatin capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (mg/kg)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mifepristone</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>AUC</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

The serum concentration of both CDB-2914 and mifepristone and their respective immunoreactive metabolites were influenced by the formulation and the route of administration. It also was noteworthy that CDB-2914, whether administered orally or intramuscularly, was characterized by slow absorption and prolonged elimination. The relatively high bioavailability of CDB-2914 was accompanied by the persistence of detectable concentrations of CDB-2914 and/or its immunoreactive metabolites, and this may reflect enterohepatic recirculation as well as the progressive production and recirculation of proximal CDB-2914 metabolites. In-vivo studies in the human and rat have demonstrated that enterohepatic recirculation of mifepristone and its metabolites occurs following oral administration (Heikinheimo et al., 1989, 1994).

In addition, adipose tissue serves as a potential reservoir for mifepristone and the monodemethylated and didemethylated metabolites in both species (Heikinheimo et al., 1989, 1994), and this may well be the case for CDB-2914 and its proximal metabolites. The re-entry and redistribution of CDB-2914 and its early metabolites into the vasculature may partially account for the sustained serum concentrations of CDB-2914 equivalents seen in this study, regardless of the route of administration.

Although the metabolites of CDB-2914 were not isolated and characterized in the present study, it appears likely that two of the earliest metabolites would be the monodemethylated and didemethylated products, based on an analogy to mifepristone (Heikinheimo et al., 1989, 1994). Noteworthy, the proximal metabolites of mifepristone were biologically active to varying degrees in the rat (Deraedt et al., 1985). The monodemethylated and didemethylated derivatives of CDB-2914 have been chemically synthesized and tested. The synthetic monodemethylated metabolite of CDB-2914 exhibited considerable affinity for the rabbit uterine PR and thymic GR, as well as antiprogestational, anti-ovulatory and postcoital antifertility activity in the rat (unpublished data).

The absorption of orally dosed mifepristone has been shown to be rapid in humans (Laitteenmäki et al., 1987; Kekkonen et al., 1996) and rats (Deraedt et al., 1985; Heikinheimo et al., 1994), and this was also observed in female rhesus monkeys in the present study. In contrast, results from the earlier of these studies (Deraedt et al., 1985) described slow and irregular absorption of mifepristone in the cynomolgus monkey. However, a different vehicle [polyethylene glycol (PEG) 300] was employed in this study, and this may account for the difference seen between rhesus and cynomolgus monkeys. In contrast, the absorption of CDB-2914 was slower than mifepristone following administration in either gelatin capsules or in an ASV, as indicated by the time to Cmax. The difference in absorption of the two antiprogestins given in these two formulations may reflect the solubility and the extent of aggregation of drug particles in the gastrointestinal tract. The AUC0-120h values for CDB-2914 and mifepristone administered orally in gelatin capsules were only 67% and 38% respectively that of the AUC values when these drugs were given in ASV.

Interestingly, the oral bioavailability of mifepristone ranged from 15% in cynomolgus monkeys to 40% in humans and rats when administered in PEG 300 (monkeys), tablets (humans) and methylacetamide (rats) respectively (Deraedt et al., 1985). In addition, when the AUC for CDB-2914 in ASV or gelatin capsule were compared with the AUC for mifepristone in ASV or gelatin capsule, the serum concentrations of CDB-2914 were 4.7- and 5.3-fold greater than those of mifepristone. Hence, CDB-2914 appeared to be more efficiently absorbed than did mifepristone in the rhesus monkey, regardless of the oral dosing formulation.
metabolites was calculated for this study in rhesus monkeys (data not shown), the value was identical to the 15 h value reported earlier (Deradaet et al., 1985) for cynomolgus monkeys. In human subjects, orally dosed mifepristone exhibits a longer elimination half-life that ranges from 25 to 30 h (Heikinheimo et al., 1989; Kekkonen et al., 1996). The longer half-life in humans appears to be the result of mifepristone binding by serum AAG rather than extensive extravascular diffusion, thereby sequestering the drug in the central compartment for a longer period. The most likely explanation for the difference between humans and laboratory species is the lack of a high-affinity serum binding protein for mifepristone in rats and monkeys (Moguilewsky and Philibert, 1985; Heikinheimo et al., 1994). Based on our preliminary serum protein binding studies, the same appears to hold true for CDB-2914.

Because of the structural similarity of CDB-2914 to mifepristone, it was considered likely that the slow elimination and increased bioavailability of CDB-2914 in the rhesus monkey might reflect high-affinity binding to one or more serum binding proteins. On the contrary, the current studies did not detect high-affinity binding of either CDB-2914 or mifepristone in rhesus monkey serum. The results for mifepristone agree with those published earlier (Moguilewsky and Philibert, 1985), which suggested that there was no specific binding of mifepristone by cynomolgus monkey serum. AAG is present in monkey serum, but the data suggest that its steroid binding characteristics differ from that of human AAG. In this regard, the results of the present study and other studies carried out using equilibrium dialysis demonstrated that purified human AAG and diluted human serum (unpublished data) bind CDB-2914, but to a lesser extent than mifepristone. Neither SHBG nor CBG appears to serve as a serum carrier protein for CDB-2914 or mifepristone in monkeys or in humans. The lack of mifepristone binding to human SHBG and CBG has been reported previously (Heubner et al., 1985; Heikinheimo et al., 1987b). Thus, the pharmacokinetics of CDB-2914 and mifepristone do not appear to be influenced by high-affinity binding to specific serum proteins in the rhesus monkey. The lack of specific binding by monkey AAG, SHBG and CBG suggests that the pharmacokinetics of CDB-2914 and mifepristone in the rhesus monkey may differ significantly from that in the human.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Lisa Radler, Richard Scott, Janet Burgenson, Sandra Propst and Bruce Till. We also thank Dr Sheri Hild for editorial assistance, and Dr Stephanie Krasnow, Dr Marisa St Claire, Steve Harbaugh, Jeff Harbaugh and Marsha Sowers for the treatment and handling of the rhesus monkeys. BIOQUAL, Inc., carried out these studies under contracts N01-HD-1-3130 and N01-HD-6-3259 to the Contraception and Reproductive Health Branch, NICHD.

References


Received on October 25, 1999; accepted on January 31, 2000.