ABSTRACT:
The metabolism of two newer antiprogestational agents, lilopristone and onapristone, was investigated using human liver microsomes, and evidence was obtained supporting a principal role of cytochrome P450 (CYP) 3A4 in their N-demethylation. Kinetic studies with microsomes from three organ donors indicated lack of biphasic kinetics at substrate concentrations up to 200 μM, consistent with a single enzyme mediating the oxidations. Selective chemical inhibitors of CYP1A2 (furafylline), CYP2C9 (sulfaphenazole), CYP2D6 (quindine), and CYP2A6/2E1 (diethyldithiocarbamic acid) did not affect initial rates of metabolism of either steroid. Gestodene and triacetyloleandomycin (selective for CYP3A enzymes) inhibited the demethylations of both antiprogestins by up to 77%. Rabbit polyclonal antibodies to CYP3A4 decreased initial rates of N-demethylation of the antihormones by up to 82%, whereas antibodies to CYP2C9 were not inhibitory. Collectively, these data thus suggest potential drug–drug interactions of these promising new therapeutic agents with concomitantly administered CYP3A4 substrates.

Antiprogestins are a relatively new class of therapeutic agents with significant promise in the treatment of some forms of breast (1, 2) and prostate (3) cancer, meningioma (4), uterine leiomyoma, and endometriosis (5). They may also be effective contraceptive agents (6, 7). The first developed antiprogestin mifepristone (RU 486) is also a potent antiadrenal corticosteroid, a characteristic that may prove unfavorable upon long-term administration of the compound. Lilopristone (ZK98.734) and onapristone (ZK98.299) (fig. 1) reverse dexamethasone-induced tyrosine aminotransferase activity in rat hepatoma cells with roughly 5% and 4% the activity of mifepristone, respectively (8), and thus represent potentially more specific antiprogestin therapy. Onapristone is also of interest due to inverted stereochemistry at the 13- and 17-positions relative to lilopristone and mifepristone, imparting different conformations to the C- and D-rings of the steroid nucleus and divergent three-dimensional structure. This could explain apparent lack of binding to orosomucoid (an α1-acid glycoprotein) in humans (9), a characteristic of mifepristone that is saturable at therapeutic doses and results in nonlinear pharmacokinetics (with dose) and a t½ of ~30 hr (10).

We recently demonstrated a principal role of CYP3A4 in the oxidative metabolism of mifepristone in human liver microsomes (11). The modest structural differences between these newer compounds and mifepristone apparently alter significantly their binding to the glucocorticoid receptor and orosomucoid (in the case of onapristone). Despite modified interactions with these two proteins, we hypothesize that lilopristone and onapristone (like mifepristone) are substrates of CYP3A4 due to the relatively promiscuous active site of this enzyme, which allows it to accommodate structurally diverse molecules. In this work, data from CYP selective chemical and immunoinhibition studies are presented that support this hypothesis and, as a result, suggest consideration of potential drug–drug interactions upon long-term administration of the antihormones.

Materials and Methods

Chemicals and Specimens. Lilopristone, onapristone, their N-demethylated metabolites, and gestodene were kindly supplied by Schering AG (Berlin, Germany). DDC, NADPH, progesterone, quinidine, sulfaphenazole, and TAO were purchased from Sigma Chemical Co. (St. Louis, MO). Furafylline was obtained from Research Biochemicals International (Natick, MA). Rabbit polyclonal antibodies specific to CYP3A4 and CYP2C9 were a generous gift from Dr. Steven A. Wrighton (Eli Lilly & Company, Indianapolis, IN). Microsomes were prepared by homogenization and differential centrifugation of nontransplantable liver from a 53-year-old male (HL-01), a 5-year-old male (HL-02), and a 36-year-old female (HL-03)—all of whom had died as a result of head trauma. The microsomes were stored until use at ~80°C in 10 mM Tris-acetate (pH 7.4) with 1 mM EDTA and 20% (w/v) glycerol. Protein (12) and CYP (13) concentrations were determined by standard methods.

Assay for the Antiprogestins and Their Metabolites. A previously described HPLC assay for the determination of mifepristone and its metabolites in serum (14) was modified for measuring lilopristone, onapristone, and their metabolite levels in microsomal incubations. Briefly, the mobile phase was methanol:acetone:water (35:30:35) at 1 ml/min through a Zorbax C18 (5 mm × 4.6 mm i.d. × 250 mm) column with UV monitoring (315 nm). Under these conditions lilopristone, onapristone, their respective monodemethylated metabolites, and progesterone (the internal standard) eluted with respective retention times of 13.3, 11.7, 7.6, 5.8, and 22.4 minutes. The autoinjector, pump, and detector were Shimadzu models SIL-9A, LC-600, and SPD-6A, respectively. A Hewlett-Packard 3392A integrator was used. Quantitation was effected with extinction coefficients from synthetic standards.
Incubation Conditions. Incubations were conducted with 0.3 mg protein/ml in 0.1 M Na₂HPO₄ buffer (pH 7.4) at 37°C, with substrate and inhibitors added in methanol (final concentration: ≤2%, v/v). Reactions were initiated by adding NADPH in buffer (to 1 mM) after 5 min preincubation, quenched after 2 min by adding a 2-fold volume of acetonitrile containing the internal standard, and vortexed. Precipitated proteins were pelleted by centrifugation (5 min at 11,000g) and 100–150 μl of the supernatant subjected to HPLC analysis.

To evaluate mechanism-based or quasi-irreversible (TAO) inhibitors, catalysis-dependent inactivation was initiated by addition of NADPH in buffer (to 1 mM) after 5 min preincubation, quenched after 2 min by adding a 2-fold volume of acetonitrile containing the internal standard, and vortexed. Precipitated proteins were pelleted by centrifugation (5 min at 11,000g) and 100–150 μl of the supernatant subjected to HPLC analysis.

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Data Analysis. For characterization of metabolite formation, substrate concentration was varied up to 200 μM and kinetic parameters estimated by nonlinear regression analyses (Minim 3.0.8) assuming single enzyme Michaelis-Menten kinetics, with a weighting factor equal to the reciprocal of the observed initial rate. All results are presented as the means of duplicate determinations ± half the range.

Results and Discussion

Preliminary experiments with the two antihormones revealed the mono-N-demethylated derivatives to be their major metabolites in microsomal incubations, with smaller amounts of didemethylated metabolites detectable after extended incubation periods. We observed very short periods of linear product formation (≤3–4 min) and hence used 2-min incubations to ensure conditions of linearity. Figure

![Chemical structures of mifepristone (RU 486, the first synthesized antiprogestin), lilopristone, and onapristone.](image)

**FIG. 1.** Chemical structures of mifepristone (RU 486, the first synthesized antiprogestin), lilopristone, and onapristone.

![Nonlinear regression analysis for lilopristone demethylation (in microsomes from HL-03) vs. substrate concentration assuming single-enzyme Michaelis-Menten kinetics and (B) Eadie-Hofstee transformation of the data. (C and D) Representative fit and Eadie-Hofstee plot for onapristone demethylation in microsomes from HL-01.](image)

**FIG. 2.** (A) Nonlinear regression analysis for lilopristone demethylation (in microsomes from HL-03) vs. substrate concentration assuming single-enzyme Michaelis-Menten kinetics and (B) Eadie-Hofstee transformation of the data. (C and D) Representative fit and Eadie-Hofstee plot for onapristone demethylation in microsomes from HL-01.

Each data point represents the mean of initial rates of antiprogestin demethylation determined in duplicate.

![Effects of antibodies to CYP3A4 and CYP2C9 on antiprogestin demethylations.](image)

**FIG. 4.** Effects of antibodies to CYP3A4 and CYP2C9 on antiprogestin demethylations.

Polyclonal antibodies to CYP3A4 and CYP2C9 (at 200 μl/mg and 75 μl/mg microsomal protein, respectively) were incubated with microsomes from HL-02 (A) and HL-03 (B) for 30 min at 25°C to evaluate their effects on lilopristone and onapristone demethylations. Substrate concentration was 100 μM, and each bar represents the mean of duplicate determinations ± half the range.

S. A. Wrighton, personal communication.
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TABLE 1

| Kinetic parameters for the demethylations of lilopristone and onapristone in human liver microsomes |
|---|---|---|---|---|---|
| | Lilopristone | | Onapristone |
| | $K_m$ | $V_{max}$ | $V_{max}/K_m$ | $K_m$ | $V_{max}$ | $V_{max}/K_m$ |
| | $\mu$M | pmol/min $\cdot$ mg protein | $\mu$l/min $\cdot$ mg protein | $\mu$M | pmol/min $\cdot$ mg protein | $\mu$l/min $\cdot$ mg protein |
| HL-01 | 5.8 | 1270 | 219 | 20.2 | 1610 | 80 |
| HL-02 | 9.9 | 2320 | 234 | 23.3 | 2770 | 117 |
| HL-03 | 10.7 | 2310 | 216 | 14.8 | 4100 | 277 |
| Mean ± SD | 8.8 ± 2.6 | 1,970 ± 600 | 220 ± 10 | 19.6 ± 4.5 | 2,830 ± 1,250 | 160 ± 100 |

2A depicts a representative fit assuming single-enzyme Michaelis-Menten kinetics, and Eadie-Hofstee transformation of the data (fig. 2B) reveals a clear lack of biphasic kinetics at concentrations up to 200 $\mu$M. This was also observed for onapristone demethylation (fig. 2, C and D) and with the microsomes from the liver donor (data not shown). Table 1 summarizes the estimated kinetic parameters and the calculated intrinsic clearances ($V_{max}/K_m$) via demethylation for each compound. Rate of elimination, as assessed by the relative intrinsic clearance values, was higher for lilopristone demethylation with the exception of microsomes from HL-03. To our knowledge, no data have been published descriptive of the in vivo biotransformations of these antihormones in humans. In experimental species, these two compounds are mainly metabolized by mono- $N$-demethylation (15). Our in vitro results suggest that this is likely the major route of metabolism in humans as well. Unlike mifepristone, which is also metabolized via hydroxylation of its 17-$alpha$-propyl moiety, the respective 17$\alpha$ and 17$\beta$ substituents of lilopristone and onapristone possess hydroxyl groups at their termini and, as might be expected, apparently do not undergo oxidation at these positions.

The CYP3A4-selective inhibitors, gestodene and TAO dose-dependently, potently and significantly reduced initial rates of lilopristone demethylation (fig. 3A) with respective IC$50$ values of 3.3 and 7 $\mu$M. Gestodene at 10 and 25 $\mu$M inhibited onapristone demethylation by 54 and 75%, respectively (fig. 3C).

Sulfaphenazole (up to 50 $\mu$M) and quinidine (up to 25 $\mu$M), competitive inhibitors of CYP2C9 and CYP2D6, respectively, were coincubated with 10 $\mu$M lilopristone and onapristone (at or below the apparent $K_m$ for their demethylations). The two inhibitors had no effect on the demethylation of either antiprogestin (data not shown). Similarly, the mechanism-based inhibitors of CYP1A2 and CYP2A6/2E1, furafylline, and DDC did not inhibit either demethylation (fig. 3, B and C) under conditions previously demonstrated to inhibit their respective enzymes maximally (16).

Antibodies to CYP3A4 were used at a concentration previously determined to inhibit the enzyme maximally and resulted in the inhibition of lilopristone and onapristone demethylations by 70 and 82%, respectively (fig. 4). Antibodies to CYP2C9 were also evaluated because of an earlier report suggesting potential CYP2C subfamily involvement in mifepristone metabolism in the rat (17). These antibodies were not inhibitory (fig. 4) at a concentration known to repress maximally CYP2C9 catalyzed tolbutamide hydroxylation by 75%, consistent with the lack of inhibition by sulfaphenazole.

Despite the structural differences with mifepristone that seem to influence several characteristics of these newer agents, the present study demonstrates that they are also principally metabolized by CYP3A4. In addition, it is extremely likely that their second demethylations are also CYP3A4-mediated, as we previously demonstrated for mifepristone (11). Because inhibition of the demethylations by gestodene, TAO, and antibodies to CYP3A4 was maximal at roughly 70 to 80%, it is possible that other non-3A subfamily CYPs may catalyze the reactions as well, but perhaps at a significantly lower rate (i.e., higher apparent $K_m$ and lower $V_{max}$—resulting in the observed monophasic kinetics). It is very probable, however, that CYP3A4 would be the clinically important site of potential drug–drug interactions due to its dominant role in the metabolism of these antihormones and numerous other xenobiotics.

References


