Methadone: A Substrate and Mechanism-Based Inhibitor of CYP19 (Aromatase)

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ABSTRACT:
The peripheral conversion of testosterone to estradiol by aromatase is the primary source of endogenous estrogen in postmenopausal women. Studies indicating that placental aromatase is able to metabolize methadone to its primary metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), led us to test the hypothesis that methadone is able to act as an inhibitor of aromatase. Using recombinant human CYP19, we examined the ability of methadone to bring about either irreversible or mechanism-based inhibition of the conversion of testosterone to estradiol. To test for reversible inhibition, racemic methadone or its metabolite EDDP or 2-ethyl-5-methyl-3,3-diphenylpyrrolidine (EMDP) was incubated for 30 min with testosterone at the $K_M$ (4 μM). To test for mechanism-based inhibition, microsomal preincubations were performed for up to 30 min using racemic methadone (1–1000 μM), R- or S-methadone (0.5–500 μM), or EDDP or EMDP (10 and 100 μM) followed by incubation with testosterone at a $V_{max}$ concentration (50 μM). Racemic methadone, EDDP, and EMDP did not act as competitive inhibitors of CYP19. Preincubation of methadone, EDDP, or EMDP with CYP19 resulted in time- and concentration-dependent inhibition, indicating a mechanism-based reaction that destroys CYP19 activity. The $K_i$ and $k_{inact}$ values for racemic methadone were calculated to be 40.6 ± 2.8 μM and 0.061 ± 0.001 min⁻¹, respectively. No stereoselectivity was observed. Methadone is metabolized by CYP19 and may act as a potent inhibitor of CYP19 in vivo. These findings may contribute to variability in methadone clearance, to drug-drug interactions, and to side effects observed in individual patients.

Methadone is a synthetic analgesic that is distinguished by its long duration of action, a property that makes it ideal for the treatment of chronic pain and for opioid withdrawal (Fredheim et al., 2008). The use of methadone is increasing, prescriptions having grown in the United States by 1300% between 1997 and 2006 (Kharasch et al., 2009), primarily as the result of its increasing use as a first-line analgesic. In addition, methadone maintenance therapy is the mainstay for the treatment of opioid addiction, but it is estimated that fewer than 10% of individuals who are addicted to heroin and prescription opioids are actually receiving methadone (Kleber, 2008).

Despite its advantages and widespread utility, the use of methadone is made difficult by complex pharmacokinetic characteristics that include a long elimination half-life and susceptibility to pharmacokinetic drug-drug interactions (Weschules et al., 2008). The goal of predictable and reproducible effective dosing is confounded by considerable interindividual variability in methadone pharmacokinetics, particularly in its clearance (up to 100-fold) (Totah et al., 2008). This variability is further complicated by stereoselectivity in methadone pharmacokinetics. Although methadone is generally administered as a racemic mixture, consisting of equal amounts of two enantiomers; the $R_-$, levo-, $l_-$, or (+) and the $S_-$, dextro-, $d_-$, or (--)methadone, these enantiomers differ in pharmacokinetic and pharmacodynamic properties. The unpredictability of the effects of methadone due to these multiple characteristics results in a high incidence of inappropriate over- and underdosing. Inappropriate dosing can clearly cause severe adverse events such as withdrawal symptoms, respiratory depression, and electrocardiographic QT interval prolongation that can result in sudden cardiac death (Krantz et al., 2003; Kalugutkar et al., 2007; Sim et al., 2007; Kharasch et al., 2009).

The dominant metabolism of methadone to its primary demethylated metabolite, EDDP, and then to its secondary metabolite, EMDP, is well documented (Fig. 1) (Pohland et al., 1971), but the enzymatic route by which these reactions occur in the body is controversial (Shiran et al., 2009). Because understanding of the metabolic pathways responsible for drug interactions or environmental effects is incomplete (Weschules et al., 2008), it is difficult to predict methadone pharmacokinetics, and adverse events during methadone therapy are often considered “idiopathic.” Patients taking methadone often have coprescriptions for many other medica-

ABBREVIATIONS: EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; EMDP, 2-ethyl-5-methyl-3,3-diphenylpyrrolidine; HPLC, high-performance liquid chromatography; P450, cytochrome P450; MIC, metabolite intermediate complex.
tions and are therefore vulnerable to drug-drug interactions (We- 

schules et al., 2008). Poorly understood drug-drug interactions may 

thus contribute to the notable increase in methadone-related deaths 

(Sims et al., 2007) that has attended the increase in the use of the 

drug over the past decade (Kharasch et al., 2009). In addition, the 
effect of methadone itself on the metabolism or pharmacokinetics 
of other medications is understudied.

Although potential interactions with methadone via CYP3A and 

CYP2B6 have been carefully studied in vitro (Iribarne et al., 1997; 

Foster et al., 1999; Wang and DeVane, 2003; Kharasch et al., 2004) 

and in vivo (Eap et al., 2002; Totah et al., 2008), it is clear that 

these enzymes cannot fully explain variability in methadone 

pharmacokinetics. Other potential routes of metabolism may be 

important. Methadone has been shown to be metabolized by aro- 
atase (CYP19), notably in the placenta (Nanovskaya et al., 2004; 

Hieronymus et al., 2006). However, drug interactions involving 
drugs that alter methadone clearance via CYP19 and interactions 

involving effects of methadone on CYP19 that might alter metab- 

olism of androgens or other drugs have not been described or tested 

for. Side effects of methadone may be explained in part by these 

interactions with CYP19. In addition, for patients with metastatic 

breast cancer who are in pain, methadone may often be copre- 

scribed with a potent aromatase inhibitor. Last, the variable phar- 

macokinetics of methadone via autoinhibition or autoinduction 

may be explained in part by its effects on aromatase.

For these reasons and as a first step toward obtaining a more 

complete understanding of methadone’s interactions with CYP19, 

we tested the ability of methadone to inhibit CYP19 under condi- 
tions in which either reversible or mechanism-based inhibition 
could be quantified.

Materials and Methods

Chemicals and Reagents. R(-)-Methadone and S(+)-methadone were 
generously provided by the National Institute on Drug Abuse Drug Supply 
Program (Bethesda, MD). 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrroline 
iodide (EDDP iodide) and 2-ethyl-5-methyl-3,3-diphenylpyrroline hydrochlo- 
ride (EMDP hydrochloride) were obtained from Alttech Associates (Deerfield, 
IL). Norgestrel was obtained from Toronto Research Chemicals Inc. (North 
York, ON, Canada). Racemic methadone hydrochloride, 17β-estradiol, testos- 
terone, β-NADP, glucose-6-phosphate dehydrogenase, and glucose 6-phos- 
phate were purchased from Sigma-Aldrich (St. Louis, MO). Monobasic and 
dibasic sodium phosphate, monobasic potassium phosphate, magnesium chlo- 
ride, high-performance liquid chromatography (HPLC)-grade acetonitrile, and 
methanol were purchased from Thermo Fisher Scientific (Waltham, MA).

Glycine (electrophoresis purity reagent) was obtained from Bio-Rad Labora- 
tories (Hercules, CA). All solutions of methadone, testosterone, 17β-estradiol, 
norgestrel, and letrozole were prepared by dissolving each compound in 
methanol and were stored at −20°C.

For recombinant human CYP19 microsomal preparations, baculovirus in- 
sect cell-expressed human CYP19 (with oxidoreductase) was purchased from 
BD Biosciences (San Jose, CA). All microsomal preparations were stored at 
−80°C until used.

Testosterone and Methadone Metabolism In Vitro by Recombinant 
CYP19. General incubation conditions were as follows. All incubations were 
performed using incubation times and protein concentrations that were within 
the linear range for reaction velocity. Testosterone and methadone were 
dissolved in methanol and diluted with the same solvent to the required 
concentrations. Any methanol in the incubation tubes was removed by drying 
in a speed vacuum before the incubation. All incubations contained recombi- 
ant human CYP19 in 100 mM sodium phosphate buffer (pH 7.4), with an 
NADPH-generating system (1.3 mM NADP, 3.3 mM glucose 6-phosphate, 3.3 

mM MgCl₂, and 0.4 U/ml glucose-6-phosphate dehydrogenase) and various 
concentrations of CYP19 substrate in a final volume of 250 μl. The final 
CYP19 concentration used was 0.01 μM when testosterone was the substrate. 
When methadone was the substrate, the P450 concentration used was 0.05 μM. 
When testosterone was the substrate, the reaction was prewarmed for 5 min at 
37°C, initiated by addition of the NADPH-generating system, and incubated at 
37°C for 10 min. When methadone was the substrate, the final incubation was 
for 30 min. All reactions were terminated by the addition of 20 μl of 60% (w/v) 
perchloric acid, followed by an immediate vortex and then by placing the tubes 
on ice.

Reversible Inhibition of Recombinant CYP19. Solutions of testosterone 
and tested inhibitor were mixed, and methanol was removed by drying in speed 
vacuum before the incubation. Incubations were then carried out in the same 
way as described in general incubation conditions except that the final testos- 
terone and CYP19 concentrations were 4 and 0.01 μM, respectively. The 
inhibition of CYP19 by methadone or letrozole was determined by measuring 
the conversion rate of testosterone to estradiol.

Mechanism-Based Inhibition of Recombinant CYP19. Recombinant 
CYP19 was preincubated with various concentrations of inhibitors. All prein- 
cubations contained tested inhibitor, 0.1 μM CYP19 and 100 mM sodium 
phosphate buffer (pH 7.4), NADPH-generating system (1.3 mM NADP, 3.3 
mM glucose 6-phosphate, 3.3 mM MgCl₂, and 0.4 U/ml glucose-6-phosphate 
dehydrogenase) in a final volume of 250 μl. Preincubinations were initiated 
and carried out in the same way as described for general incubation conditions. 
After a period of time, each 250-μl preincubation mixture was diluted 10 times 
with a solution containing testosterone, sodium phosphate buffer, and 
NADPH-generating system to reach a final incubation mixture of 50 μM 
testosterone and 0.01 μM CYP19. The dilution was to eliminate competitive 
inhibition as a possible cause of the inhibitory effect. This incubation mix was 
then incubated at 37°C for 10 min. The reaction was terminated the same way 
as described in general incubation conditions. The extent of inactivation 
of CYP19, expressed as the percentage of activity remaining after preincubation, 
was determined by measuring the conversion rate of testosterone to estradiol.

Extraction. All samples were extracted immediately after the incubation. 
First, 25 μl of norgestrel (500 mM) was added to each sample as an internal 
standard. The incubation mixture was centrifuged at 14,000 rpm for 5 min at 
room temperature. The supernatant layer was made alkaline by adding 500 μl 
of glycine-NaOH (1 M) buffer (pH 11.3) and extracted by adding 6 ml of ethyl 
acetate. This mixture was vortex-mixed for 10 s and then centrifuged at 26,000 
rpm for 15 min. The organic layer was transferred to 13 × 100-mm glass 
culture tubes and evaporated to dryness. The resulting residue was reconsti-
tuted with mobile phase (50% 10 mM monobasic potassium phosphate, 40% 
acetonitrile, and 10% methanol) and analyzed as described below.

Quantifications of Estradiol and EDDP Formation. HPLC assays with 
UV detection were developed for the quantification of testosterone conversion 
to estradiol and of methadone conversion to EDDP. The HPLC-UV system 
comprising a Waters (Milford, MA) model 510 HPLC pump and a Waters 
model 717plus autosampler, coupled with a Waters 486 tunable absorbance 
detector. The separation system consisted of a Zorbax SB-C18 column (46.5 
× 150 mm, 3.5-μm particle size; Phenomenex, Torrance, CA), a Nova-Pak C₁₈ 
Guard column (4 mm; Waters, Dublin, Ireland). An isocratic elution was used 
to separate the compounds. The mobile phase consisted of 50% 10 mM 
monobasic potassium phosphate, 40% acetonitrile, and 10% methanol without 

Fig. 1. Dominant route of methadone metabolism. Methadone is 
N-demethylated to the primary metabolite, EDDP. EDDP is further 
N-demethylated to the secondary metabolite, EMDP.
enzyme was due to inactivation. As indicated above, we fit all of the data for
during the incubation period and that loss of
The relationship between
and inhibitor concentration was fit to eq. 3:
where \( k_{\text{inact}} \) is the rate constant for inactivation, \([I]\) is inhibitor or inactivator concentration, and \( K_i \) is the inactivator concentration that produced half the maximal rate of inactivation, analogous to a \( K_m \). Equation 3 assumes that there was a negligible change in \([I]\) during the incubation period and that loss of enzyme was due to inactivation. As indicated above, we fit all of the data for each inhibitor concentration to obtain a single estimate for \( k_{\text{inact}} \) and a single estimate for \( K_i \) using eqs. 1 to 3.

**Results**

**Testosterone Metabolism by Recombinant CYP19.** To measure the activity of enzyme, the rate of aromatization of testosterone to 17\(\beta\)-estradiol was used as a marker of CYP19 activity. Under the conditions used, CYP19 was able to catalyze the generation of estradiol from testosterone with a \( V_{\text{max}} \) of 9.1 pmol/min/pmol of P450 and \( K_m \) of 4.0 \(\mu\)M (data not shown). A concentration of testosterone at the \( K_m \) was chosen to test for reversible, competitive inhibition of CYP19 by methadone. At 50 \(\mu\)M testosterone, the rate of estradiol formation was at \( V_{\text{max}} \). This concentration was therefore chosen for experiments to test the mechanism-based inhibition of CYP19 by methadone. The rate of estradiol formation was used as a measure of P450 activity.

**Reversible Inhibition of CYP19 by Methadone.** Figure 2 shows the rate of estradiol formation from 4 \(\mu\)M testosterone in the presence of a range of methadone concentrations from 0 to 2 mM. No effect of methadone on the rate of metabolism of this probe for CYP19 was observed under these conditions, even at the highest concentration of methadone used. The selective CYP19 inhibitor letrozole (1 \(\mu\)M), used as positive control, was able to completely block the formation of estradiol.

**Mechanism-Based Inhibition of CYP19 by Methadone.** Because no competitive inhibition of CYP19 was observed under the conditions used, the possibility that methadone itself could be metabolized by CYP19 under these conditions was tested. Metabolism of methadone to EDDP by recombinant human CYP19 was observed. Experiments to characterize this metabolism were then performed under conditions in which secondary metabolism from EDDP to EMMP was not detected. Figure 3 depicts the rate of EDDP generation from methadone across a range of methadone concentrations (0–2000 \(\mu\)M). These data indicate metabolism of methadone to EDDP with a \( K_m \) of 314 \(\mu\)M and a \( V_{\text{max}} \) of 7.6 pmol/min/pmol of P450. The enzyme converting methadone to EDDP was confirmed to be CYP19 by demonstrating that the conversion of methadone (1000 \(\mu\)M) to EDDP was completely inhibited by 1 \(\mu\)M letrozole (data not shown).

**Mechanism-Based Inhibition of CYP19 by Methadone.** Because methadone metabolism to EDDP could be catalyzed by CYP19, pilot experiments were conducted to test the ability of methadone to inactivate CYP19 activity in a time-dependent manner. When CYP19 was preincubated in the presence of 1 mM methadone and then incubated with a \( V_{\text{max}} \) concentration of testosterone (50 \(\mu\)M), this resulted in a log-linear time-dependent loss of CYP19 activity (data not shown). If enzyme was preincubated under the same
concentration. When 0 to 500 μM methadone was preincubated for 25 min, inhibition of 50% of activity was seen at approximately 50 μM methadone, whereas 30% inhibition was produced by the presence of 10 μM methadone (data not shown). When the individual enantiomers (0–500 μM, over 5–20 min) were separately tested under the same conditions, no stereoselectivity was observed (data not shown).

To estimate the kinetics of the interaction between methadone and CYP19 during preincubation, a range of methadone concentrations and preincubation times were tested. Figure 4A depicts the preincubation time and concentration dependence of the effect of methadone on CYP19 activity. Control incubations were performed in the presence of NADPH but without methadone. The rates of metabolism at 0 preincubation time for each concentration were consistent with the $V_{\text{max}}$ activity of CYP19 under these conditions. It was therefore possible to normalize the data at each concentration to the activity seen at 0 min of preincubation. Kinetic analyses of the rates of enzyme inactivation in the presence of methadone were performed with NONMEM, and predicted time curves for inactivation at each methadone concentration were obtained (Fig. 4A). The $K_I$ and $k_{\text{inact}}$ values were calculated to be $40.6 \pm 2.8$ μM, and $0.061 \pm 0.001$ min$^{-1}$, respectively. A close correlation between observed and predicted rates of CYP19 was observed (Fig. 4B), indicating that the data are reliably represented by the model.

Because methadone is metabolized by CYP19, we tested the possibility that inhibition of CYP19 by methadone might be due to the metabolites formed. We directly tested the metabolites (EDDP and EMDP) in both reversible and mechanism-based experimental designs in the same way as described for methadone itself. No competitive inhibition was observed when EDDP or EMDP was tested at concentrations up to 100 μM (data not shown). When mechanism-based inhibition was tested, inactivation of CYP19 was observed by EDDP or EMDP at concentrations as low as 10 μM (data not shown). Figure 5 shows that the rate of inactivation by EMDP (100 μM) was slower than that by either EDDP (100 μM) or racemic methadone (100 μM), which appeared to be equipotent.

**Discussion**

The effective use of methadone in the treatment of pain and heroin addiction is compromised by poor understanding of the metabolic routes involved in its disposition. One direct consequence is inability to anticipate drug interactions in patient populations, for whom multiple medications are commonly coprescribed. In this study, we demonstrated that CYP19 is able to metabolize methadone and that methadone is a mechanism-based inhibitor of the enzyme.

Our data showed no clear competitive inhibition of CYP19 by methadone using recombinant human CYP19. Ahmed and colleagues (Zharkova et al., 2006) reported competitive inhibition with a $K_I$ of 393 μM in human placental microsomes, but, consistent with the present data, they did not demonstrate competitive inhibition in recombinant CYP19. Although we have not yet tested placental microsomes, it may be anticipated that a lower $K_I$ would be obtained if methadone acts as a mechanism-based inhibitor in the placenta.

We observed that human recombinant CYP19 was able to metabolize methadone to the dominant metabolites that are seen in vivo, and that this metabolism can be completely blocked by the specific aromatase inhibitor letrozole. These data are consistent with those noted by Ahmed and colleagues (Nanovskaya et al., 2004), who reported methadone metabolism in human placental microsomes and by recombinant CYP19. This finding may be important. First, it is possible that CYP19 contributes to the clearance of methadone in vivo, as suggested by a number of clinical observations.
has been documented to increase during pregnancy, when placental CYP19 has high activity (Pond et al., 1985; Nanovskaya et al., 2004). It is possible that fetal CYP19 activity could be responsible in part for the methadone withdrawal observed in neonates of methadone patients (Szetó et al., 1981; Serane and Kurian, 2008). That said, the extent to which CYP19 metabolizes methadone in general is unclear, and withdrawal from methadone is often unanticipated, is idiosyncratic, and is poorly understood. Second, because the biochemical aromatization of testosterone by CYP19 is initiated by the removal of a methyl group and because CYP19 is also able to remove methyl groups from methadone, cocaine (Osawa et al., 1997), and buprenorphine (Deshmukh et al., 2003), it is possible that CYP19 is involved in the demethylation of other drugs.

We have shown that methadone is able to bring about time- and concentration-dependent inhibition of CYP19, consistent with the action of an irreversible mechanism-based or “suicide” inhibitor. The data meet established criteria (Silverman, 1988) for the involvement of methadone as a mechanism-based inhibitor of CYP19. Although the clinical relevance of this interaction is not clear, it is possible that it contributes to the variability in methadone concentrations with resultant potentially lethal consequences. Methadone accumulation does not occur universally, and multiple factors may underlie the autoinhibition observed in some patients, in contrast to the autoinduction observed in others (Morton, 2007). The poor understanding of the effects of methadone on its own clearance over time also impedes our ability to dose accurately, to anticipate withdrawal, and to manage withdrawal in multiple clinical settings. These include the withdrawal that neonates of methadone-treated mothers experience (Serane and Kurian, 2008), the withdrawal symptoms that often occur during pregnancy (Pond et al., 1985), and the withdrawal symptoms that occur with routine use of methadone to treat pain or heroin addiction.

Our data indicate that EDDP seemed to be as potent a mechanism-based inhibitor of CYP19 as methadone itself, whereas EMDP seemed to be weaker. This finding suggests that these metabolites may contribute to the inhibitory effect on CYP19, although they are present at lower concentrations than that of methadone in vivo (Kharasch et al., 2008). Mechanism-based inhibition can involve the formation of a metabolite intermediate complex (MIC) that inactivates the enzyme and takes it out of the active pool (Jones et al., 1999). Such inhibition brought about by metabolite intermediate complex formation has been demonstrated for a number of N-demethylated drugs including diltiazem (Jones et al., 1999) and erythromycin (Ortiz de Montellano et al., 1981). Compared with reversible inhibition, irreversible inhibition more frequently results in unfavorable drug-drug interactions because the inactivated P450 enzyme has to be replaced by newly synthesized protein (Kalugutkar et al., 2007), and severe clinical consequences may result (Wilkinson, 2005).

Mechanism-based inhibition can result from bioactivation to a reactive intermediate that interacts with the heme prosthetic group of a cytochrome P450 or that covalently modifies the apoprotein (Kalugutkar et al., 2007; Hollenberg et al., 2008). Although it is possible in situations in which one species is responsible for MIC formation to measure the kinetics of spectral change caused by interaction with heme, methadone and both its metabolites all seemed to be active mechanism-based CYP19 inhibitors in this study. As a result, we were not able to determine the kinetics of MIC formation by the individual species, and the precise molecular mechanism responsible for this mechanism-based inactivation requires further study.

We have shown that CYP19 is able to demethylate methadone in vitro and that this drug is a mechanism-based inhibitor of CYP19. Although the clinical relevance of this interaction is not clear, it is possible that it contributes to the variability in methadone clearance, to drug-drug interactions caused by methadone via CYP19 inhibition, and to the side effects observed in individual patients.

Fig. 5. Mechanism-based inhibition of CYP19 by racemic methadone, EDDP, and EMDP. Methadone (MTD), EDDP, and EMDP (100 μM) were preincubated with recombinant human CYP19 (0.1 μM) for 0, 5, 10, 15, or 20 min, and then activity of CYP19 (0.01 μM) was assessed in incubations carried out in duplicate (n = 2). Representative data were plotted as log percent CYP19 activity observed at zero minutes of preincubation.

MTD 100 μM
EDDP 100 μM
EMDP 100 μM
Log (% CYP19 Activity)
Preincubation Time (min)
0 5 10 15 20
1.5 1.6 1.7 1.8
2 2.1

highly hydrophobic drug in tissues and at the CYP19 active site may be substantially higher (Levine et al., 1995).

Because methadone seems to be able to inhibit CYP19, it follows that methadone may alter the metabolism and disposition of endogenous testosterone and androstenedione. Lower concentrations of estradiol and of FSH have been documented in men taking methadone (Hallinan et al., 2009), and low bone mineral density that may be due to low estrogen concentrations has been documented in 83% of patients in a methadone maintenance treatment program (Kim et al., 2006). It is conceivable that other side effects of methadone, which include flushing, muscle pain, and symptoms reminiscent of estrogen withdrawal (Senay, 1985; Bäckström, 1995), may be explained in part by the drug’s action on CYP19.

Unanticipated accumulation of methadone in patients vulnerable to autoinhibition may contribute to unexpectedly high methadone concentrations with resultant potentially lethal consequences. Methadone accumulation does not occur universally, and multiple factors may underlie the autoinhibition observed in some patients, in contrast to the autoinduction observed in others (Morton, 2007). The poor understanding of the effects of methadone on its own clearance over time also impedes our ability to dose accurately, to anticipate withdrawal, and to manage withdrawal in multiple clinical settings. These include the withdrawal that neonates of methadone-treated mothers experience (Serane and Kurian, 2008), the withdrawal symptoms that often occur during pregnancy (Pond et al., 1985), and the withdrawal symptoms that occur with routine use of methadone to treat pain or heroin addiction.

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References


