Short Communication

The In Vitro Interaction of Dexmedetomidine with Human Liver Microsomal Cytochrome P4502D6 (CYP2D6)

ABSTRACT:

The effect of dexmedetomidine (DEX) on cytochrome P4502D6 (CYP2D6)-dependent dextromethorphan O-demethylase (DEXTROase) activity was studied using native human liver microsomes. DEX (0.01–4.0 μM) inhibited DEXTROase activity (IC50 = 1.8 ± 0.25 μM) and was less potent than quinidine (QND), a prototypical and clinically relevant CYP2D6 inhibitor (IC50 = 0.22 ± 0.02 μM; mean KI = 0.07 μM). Similar results were obtained with human B-lymphoblast microsomes containing cDNA-expressed CYP2D6 (DEX, IC50 = 2.2 μM; QND, IC50 = 0.15 μM). Formal kinetic analyses indicated that DEX was a reversible mixed (competitive/noncompetitive) inhibitor of DEXTROase activity in human liver microsomes, where KI env > KI and α > 1 (KI = 0.4 ± 0.2 μM; KI env = 2.3 ± 0.9 μM; α = 8.1 ± 6.8; N = 3 livers). In addition, DEX elicited a Type IIb difference spectrum (λmax = 436 nm; λmin = 414 nm) when added to cDNA-expressed CYP2D6 under aerobic (oxidized) conditions. These data indicated that DEX was able to bind reversibly to the heme (ferric) iron of CYP2D6. It is postulated that binding occurs via the 4(5)-substituted imidazole moiety. In this instance, binding was characterized by a spectral dissociation constant (KI) of 0.4 μM that was identical to the KI obtained with native human liver microsomes.

DEX1 (Abbott-R5499), the pharmacologically active optical dextro isomer of medetomidine (fig. 1), is a novel and highly selective α2-adrenergic receptor agonist with potent sedative, analgesic, and anesthetic effects. In addition, DEX seems to provide sedation and analgesia without significant ventilatory depression (1–4). Because DEX has been shown to diminish anesthetic requirements, anesthetic recovery and perioperative anxiety, it is currently in clinical trials as a perioperative anesthetic adjuvant agent (1–4). The decreased sympathetic nervous system activity associated with DEX results in decreased plasma concentrations of norepinephrine, as well as a dose-dependent decrease in arterial blood pressure and heart rate, which leads to hemodynamic stability. Moreover, DEX may decrease the risk of adverse cardiac effects, including myocardial ischemia, which is particularly important in surgery involving patients with a compromised cardiovascular system (2).

By virtue of the fact that DEX is a 4(5)-substituted imidazole, it has the potential to inhibit CYP-dependent monoxygenase activity (5–8). This has been evidence by its inhibitory effect on a number of N-demethylation and O-dealkylation reactions in rat and human liver microsomes (9–11). In the rat, however, DEX (10–100 μg/kg) has been shown to elicit only a modest effect (≤36% increase) on hexobarbital sleeping time and has no effect on the elimination of aminopyrine in vivo (9). In the case of human liver microsomes, the concentration of DEX required to inhibit CYP1A2-dependent 7-ethoxyresorufin O-deethylation and CYP3A-dependent alfentanil O-dealkylation by 50% is 0.5 μM, and ~1.0 μM, respectively (9, 10, 12). In addition, DEX has been reported to inhibit the CYP2C9-dependent metabolism of (S)-warfarin and is considered to be a broad-spectrum human CYP form inhibitor in vitro (11).

However, data describing the in vitro interaction of DEX with human liver microsomal CYP2D6 are lacking. Interaction of DEX with CYP2D6 may be clinically relevant because the enzyme has been shown to catalyze the O-demethylation of codeine to yield morphine, and codeine is regularly used in the management of postoperative pain (13, 14). Furthermore, CYP2D6 is also involved in the metabolism of a large number of other drugs, including imipramine, desipramine, encainide, propranolol, thioridazine, and amitryptiline (15). Therefore, the purpose of this study was to characterize the interaction of DEX with CYP2D6 using native human liver microsomes and human B-lymphoblastoid microsomes containing the cDNA-expressed form of the enzyme.

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1 Abbreviations used are: DEX, dexmedetomidine; IC50, concentration of DEX required to inhibit activity by 50%; KI, apparent inhibition constant; IC50 min, concentration of free DEX (drug nominally in equilibrium with enzyme); IC50 max, concentration of total DEX added to the cuvette; IC50 max, concentration of drug required to inhibit activity by 50%; KI env, apparent dissociation constant of the enzyme-inhibitor-substrate, or EIS, complex; SERT, sertraline; FLUV, fluvoxamine.
Materials and Methods

Chemicals. (QND (97% purity) was obtained from the Aldrich Chemical Co. (Milwaukee, WI). [O-Methyl-14C]DEXTRO (51 mCi/mmol; >99% purity) was synthesized as previously described and was dissolved in ethanol (16). [14C]HCHO (30.1 mCi/mmol) was purchased from Amersham International (Arlington Heights, IL). Activated charcoal (untreated powder, 100–400 mesh; catalog no. C-5260; lots no. 54H0279) was obtained from the Sigma Chemical Co. (St. Louis, MO). The charcoal was freshly prepared as a suspension (0.8% w/v) in 0.1 M potassium phosphate buffer (pH 7.4) and was stirred continuously while in use. All other reagents were purchased commercially at the best obtainable grade. DEXTRO was dissolved in ethanol (99.8% purity) was obtained from Dr. K. Marsh (Abbott Laboratories, North Chicago, IL). Human B-lymphoblast microsomes containing cDNA-expressed wild-type CYP2D6-Val74 (0.53 nmol CYP/mg) were purchased from Gentest Corp. (Woburn, MA).

Preparation of Human Liver Microsomes. Human liver tissue (subject HFO, GC4, FRX, HAK, ICF, and GEQ) was obtained from the International Institute for the Advancement of Medicine (Exton, PA). The liver tissue was chopped into small (6.0 g) pieces and finely minced with a hand-held razor blade. Thereafter, microsomes were prepared by differential centrifugation (16).

DEXTROase Activity. Incubations were conducted in 2.0 ml polypropylene microcentrifuge tubes (ClickSeal microtubes, Research Products International Corp., Mount Prospect, IL) in a Dubnoff shaking water bath at 37°C (under air). All procedures were conducted as previously described (16), except that the methanol extraction step was omitted and replaced with charcoal.

Briefly, the final assay volume was 0.5 ml and consisted of the following at the indicated final concentration: 0.1 M potassium phosphate buffer (pH 7.4), EDTA (0.1 mM), MgCl2 (3.0 mM), microsomal protein (0.5 mg/ml) and [O-methyl-14C]DEXTRO (2.5–20 μM; 51 mCi/mmol). After a 3-min preincubation period, the reaction was initiated by addition of a NADPH-generating system containing NADP+ (4.0 mM), D-glucose 6-phosphate dehydrogenase (Sigma type VII, from Baker’s yeast, 1.8 units/ml), and the reaction was continued for 2 min at 37°C. The mixture was extracted with 100 μl of 3 M NaOH. After thorough vortexing, the charcoal suspension (0.45 ml) was added, and the sample was vortexed once again. All samples were immediately centrifuged at 4°C (16,000g for 10 min). Thereafter, 0.5 ml of supernatant was mixed with 15 ml of Hionic-Fluor scintillation cocktail (Packard Instrument Co., Meriden, CT), and the samples were analyzed for [14C]HCHO by radioassay (16). The recovery of [14C]HCHO in the supernatant was 88 ± 3.8%. A number of incubations were conducted in the absence of the NADPH-generating system to determine the level of unmetabolized [O-methyl-14C]DEXTRO remaining in the supernatant (≈10%). DEXTROase activity was determined as described previously (16). Briefly, the quantity of formaldehyde formed was calculated from the net disintegrations per minute observed (sample minus no NADPH blank disintegrations per minute), corrected for formaldehyde recovery. The total corrected disintegrations per minute were then converted to nanomoles of product from the specific activity of the substrate.

The five human livers used in this study (subject HFO, GC4, FRX, HAK, and ICF) exhibited the extensive metabolizer phenotype (DEXTROase Vmax/KM ≥ 1.0 ml/hr/mg), characterized by an apparent KM of 8.7 ± 0.1 μM, Vmax of 125 ± 11.1 ml/hr/mg, and Vmax/KM of 1.3 ± 0.3 ml/hr/mg (16). In addition, ketoconazole (≥10 μM) had no effect on DEXTROase activity in this set of microsomes (data not shown). Some experiments were performed with microsomes of subject GEQ, which lacked immunologically detectable CYP2D6 protein, where DEXTROase activity was insensitive to QND (≥10 μM) and conforming to the poor metabolizer phenotype (Vmax/KM < 0.01 ml/hr/mg) (16).

Incubations with human B-lymphoblastoid microsomes containing overexpressed wild-type CYP2D6 were conducted as described for native human liver microsomes, except that the reactions were started by addition of rapidly thawed microsomal protein (final assay concentration was 0.2 μg/ml; 105 pmol CYP2D6/ml) (16).

Optical Difference Spectroscopy. Binding spectra were conducted with human B-lymphoblast microsomes containing cDNA-expressed CYP2D6. Spectra were recorded under oxidized aerobic conditions at room temperature, using a model UV-2101PC UV/visible scanning spectrophotometer (Shimadzu Scientific Instruments, Inc., Wood Dale, IL). Microsomes were diluted with 0.1 M potassium phosphate buffer (pH 7.4), containing glycercol (20%, v/v), to a final CYP concentration of 0.5 mM/0.95 mg protein/ml. The diluted microsomal suspension was equally divided between two 1.0 ml quartz cuvettes (1-cm path length), and a baseline was recorded (500 nm to 350 nm). Aliquots (1 μl) of an aqueous solution of DEXTRO (final concentration range: 0.4–4.0 μM) were added to the sample cuvette, whereas an equal volume of water was added to the reference cuvette, and a difference spectrum was scanned from 500 nm to 350 nm (17, 18). During the spectral titration of CYP2D6 with DEXTRO, the total amount of water added (≥10 μl; ±10%, v/v) was relatively small and did not substantially increase the final volume in each cuvette. Binding to CYP2D6 was monitored as the absorbance difference (ΔA) between the wavelength pair 436 nm (maxima) and 414 nm (minima) (17). Kd and the maximal extent of binding (ΔAmax/0.2 nmol CYP) were determined by linear graphical methods: 1) a double-reciprocal plot of 1/ΔA vs. 1/[DEXTRO], and 2) a Scatchard plot of ΔA/[DEXTRO] vs. ΔA (17, 18). Preliminary analysis of the data indicated that Kd ∼ [CYP]. Therefore, to account for ligand depletion effects, data were reanalyzed based on the concentration of [DEXTRO]. At each concentration of DEXTRO added to the cuvette ([DEXTRO]total), [DEXTRO]free was calculated using eq. 1:

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[\text{DEXTRO}]_{\text{free}} = [\text{DEXTRO}]_{\text{total}} - \frac{\Delta A \cdot [\text{CYP}]}{\Delta A_{\text{max}}},
\]

In this instance, \(\Delta A_{\text{max}}\), represents uncorrected maximal extent of binding (0.077/nmol CYP) and was determined graphically using values of \([\text{DEXTRO}]_{\text{free}}\).

Inhibition Studies. The inhibition of DEXTROase activity was studied with both DEXTRO and QND dissolved in methanol, such that the final concentration of methanol in the assay did not exceed 0.5% (v/v). Methanol elicits only a minimal effect (≤10% inhibition) on DEXTROase activity. Initial experiments were conducted to determine IC50. In these experiments, the final concentration of DEXTRO approximated apparent KM. When substrate concentration ∼ KM, then IC50/2 ∼ Kd for a competitive inhibitor (19). IC50 was obtained from a plot of percentage activity remaining (relative to a methanol alone incubate) vs. log10 of DEXTRO or QND concentration (0.01–4.0 μM).

Estimates of inhibition constant(s) and type of reversible inhibition were determined using both Dixon (1/initial rate vs. inhibitor concentration) and Cornish-Bowden (DEXTRO concentration/initial rate vs. inhibitor concentration) plot procedures (20, 21). The former yielded estimates of Kd, whereas the Cornish-Bowden plot yields a value of Kd. Mixed (competitive/ noncompetitive) inhibition was confirmed using a secondary plot of Dixon plot slope vs. 1/[DEXTRO] concentration (20). For a mixed (competitive/ noncompetitive) inhibitor, Kd ≥ K, and α > 1 (α = Kd/K). At least three concentrations of DEXTRO were used in the analysis and encompassed the range 2.5 μM (≈Kd/4) to 20 μM (≈2 Kd). At each concentration of DEXTRO, the concentration of QND (0.2, 0.1, 0.05, and 0 μM) and DEXTRO (4.0, 2.0, 1.0, 0.5, 0.2, 0.1, and 0 μM) was varied.

Results and Discussion

In the presence of native human liver microsomes, both DEXTRO and QND (0.01–4.0 μM) inhibited DEXTROase activity in a concentration-dependent manner (fig. 2, A and B). However, QND (IC50 = 0.22 ± 0.02 μM; mean ± SD; N = 3 livers) was a more potent inhibitor of DEXTROase activity than DEXTRO (IC50 = 1.8 ± 0.25 μM; mean ± SD; N = 5 livers). This finding was confirmed with human B-lymphoblastoid microsomes containing cDNA-expressed CYP2D6 (DEXTRO, IC50 = 2.2 μM; QND, IC50 = 0.15 μM) (fig. 2C). Additional experiments with microsomes prepared from the liver of subject GEQ indicated that DEXTRO was also able to inhibit DEXTROase activity (IC50 ∼ 0.5 μM) in the absence of CYP2D6 (data not shown). Because DEXTROase activity in GEQ microsomes was insensitive to QND, and was inhibited by ketoconazole (≤10 μM), it was likely that DEXTRO inhibited CYP3A-dependent DEXTROase activity in this set of microsomes. In fact, the IC50 obtained was comparable with that...
reported for the inhibition of CYP3A-dependent alfentanil metabolism in human liver microsomes (IC₅₀ = 0.4–1.0 μM) (10, 12).

Formal kinetic analysis confirmed that QND was a potent competitive inhibitor (mean Kᵢ = 0.07 μM) of CYP2D6 (table 1). These data compared favorably with previously reported Kᵢ values (0.03–0.2 μM) (23–26). In contrast to QND, DEX behaved as a mixed (competitive/noncompetitive) inhibitor (fig. 3), characterized by a mean Kᵢ and K_{ies} of 0.4 μM and 2.3 μM, respectively (table 1) (19–22).

The difference spectrum obtained after addition of DEX to human B-lymphoblastoid cell microsomes containing cDNA-expressed wild-type CYP2D6-Val³⁷⁴ is presented in fig. 4A. The presence of a trough at ~415 nm and peak at ~435 nm was indicative of a Type IIb spectral perturbation (17, 18). In agreement with the observations of others (11), a similar difference spectrum (λ_{max} = 433 nm; λ_{min} = 409 nm) was also obtained with native human liver microsomes (data not shown). Such a spectral perturbation is consistent with direct and reversible binding of the drug to the oxidized (ferric) heme iron of CYP, and it is postulated that binding occurs via the 4(5)-substituted imidazole moiety (5–8, 17, 18). Because the abundance of CYP2D6 in native human liver microsomes is relatively low (<5% of total CYP) (27), a spectral titration was performed with cDNA-expressed CYP2D6 over a DEX concentration ([DEX]_{free}) range of 0.2–3.6 μM.

In this instance, binding to the protein was measured as an absorbance difference (ΔA₄₃₆nm–₄₁₄ nm) and was saturable at concentrations above 1.5 μM (fig. 4A). Both double-reciprocal and Scatchard plots (fig. 4B) indicated that binding was monophasic, and yielded a Kₛ of

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**TABLE 1**

<table>
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<th>Parameter</th>
<th>Subject ID</th>
<th>Mean ± SD</th>
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<tr>
<td>K_{ies} (µM)</td>
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</tr>
<tr>
<td>α</td>
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<td>16</td>
</tr>
<tr>
<td>QND Kᵢ (µM)</td>
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**FIG. 2.** Inhibition of DEXTROase activity by QND or DEX. The inhibitory effect of (A) DEX and (B) QND was studied with native human liver microsomes. The mean (±SD) IC₅₀ value for DEX and QND was 1.8 ± 0.25 μM (N = 5 livers) and 0.22 ± 0.02 μM (N = 3 livers), respectively. (C) The effect of DEX (closed circles) and QND (open circles) on DEXTROase activity catalyzed by human B-lymphoblastoid microsomes containing cDNA-expressed CYP2D6 was also investigated (DEX, IC₅₀ = 2.2 μM; QND, IC₅₀ = 0.15 μM). In all cases, data are expressed as the percentage activity remaining (relative to a methanol alone/control incubate).

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**FIG. 3.** Inhibition of human liver microsomal DEXTROase activity by DEX. Representative (A) Cornish-Bowden and (B) Dixon plot. (Inset) Secondary plot of Dixon plot slope vs. 1/[DEX]. Data were consistent with mixed (competitive/noncompetitive) inhibition. The effect of DEX (0.1–4.0 μM) on DEXTROase activity was studied at a final DEXTRO concentration of 5.0 μM (closed circles), 10 μM (open circles), and 20 μM (closed triangles).
plasma is low (27). In vivo CYP2D6 shown to be characterized by a similar binding of DEX to native human liver microsomes has been reported to be characterized by a $K_i$ of ~0.1 μM (28). Interestingly, the binding of DEX to native human liver microsomes has been shown to be characterized by a similar $K_i$ (~0.3 μM), although biphasic binding ($K_{d1}$ ~4.3 μM) was detected over a wider DEX concentration range (0.1–29 μM) (11). However, interpretation of these data is complicated by the fact that multiple CYP forms are present in native human liver microsomes (27).

Based on the results of this study, it is concluded that DEX is a relatively potent inhibitor of CYP2D6, although it is less potent than QND (~6-fold higher $K_i$). Because DEX has already been shown to be a CYP1A, CYP3A, and CYP2C9 inhibitor in human liver microsomes (9–11), it does seem that the drug is a broad-spectrum CYP form inhibitor in vitro. However, it can be argued that the effect of DEX on human liver microsomal CYP2D6 is clinically irrelevant, assuming that the concentration of total (free and protein bound) drug in plasma equates with the concentration of drug in hepatic tissue (19). For instance, at therapeutic doses ([QND]plasma ~1.0 μM), QND is a very potent inhibitor of CYP2D6 ([QND]plasma/$K_i$ ~14.3). By comparison, SERT ([SERT]plasma/$K_i$ ~0.15) and FLUV ([FLUV]plasma/$K_i$ ~0.33) are both relatively weak inhibitors of CYP2D6 in vivo (19). Therefore, because the concentration of DEX in plasma is low (~0.5 ng/ml; ~4 nM at steady state), it is anticipated that the clinical interaction of DEX with CYP2D6 will be minimal ([DEX]plasma/$K_i$ ~0.01). In fact, this may hold true for most of the CYP forms present in human liver. In accordance, DEX has already been shown to inhibit CYP3A (alfentanil N-dealkylation) in vitro (IC$_{50}$ ~0.5–1.0 μM) (10, 12), but fails to alter significantly the pharmacokinetics of midazolam in vivo. Midazolam is also a CYP3A substrate and undergoes extensive metabolism (~75% of the dose) via the 1’-hydroxylation pathway (29, 30).

$\Delta A_{\text{max}} = 0.072$ nmol CYP2D6) that was identical to $K_i$ (table 1). The correlation between $K_i$ and $K_{d1}$ was not unexpected, given that binding of QND to cDNA-expressed CYP2D6 has been shown to be characterized by a similar $K_i$ (~0.3 μM), although biphasic binding ($K_{d1}$ ~4.3 μM) was detected over a wider DEX concentration range (0.1–29 μM) (11). However, interpretation of these data is complicated by the fact that multiple CYP forms are present in native human liver microsomes (27).

FIG. 4. Spectral titration of cDNA-expressed CYP2D6 with DEX.

(A) An absorbance difference ($\Delta A_{\text{max}}$) was measured over a [DEX]$_{\text{free}}$ range of 0.2–3.6 μM in the presence of human B-lymphoblastoid microsomes containing cDNA-expressed CYP2D6-Val$^{374}$ (0.5 nmol CYP/ml). Microsomes were diluted in 0.1 M potassium phosphate buffer (pH 7.4) containing glycerol (20%, v/v). (Inset) Difference spectrum recorded after the addition of DEX (2.8 μM). (B) Double-reciprocal plot (1/ΔA vs. 1/[DEX]$_{\text{free}}$) of data. (Inset) Scatchard plot ($\Delta A$/[DEX]$_{\text{free}}$ vs. ΔΔ). Both linear plots yielded a $K_i$ of 0.4 μM ($\Delta A_{\text{max}}$ nmol CYP2D6 = 0.072).

References


