Ketamine as a Probe for Medetomidine Stereoisomer Inhibition of Human Liver Microsomal Drug Metabolism

Evan D. Kharasch, M.D., Ph.D.,* Susan Herrmann, B.S.,† Rita Labroo, Ph.D.‡

Medetomidine (MDM) is a novel, selective, α2 adrenergic agonist with potent sedative, hypnotic, and analgesic properties, currently undergoing evaluation as an anesthetic adjuvant. The pharmacologic effects of MDM are stereospecific, due entirely to the D-isomer (MDM), whereas the L-isomer (LMED) is essentially inactive. MDMD, a 4(5)substituted imidazole, has been shown to inhibit adrenal steroidogenesis and human liver microsomal alfentanil metabolism, reactions mediated by cytochrome P-450. The mechanism of MDM inhibition of cytochrome P-450 is unknown. The purpose of this investigation was to determine the mechanism of MDM inhibition of human cytochrome P-450-mediated microsomal metabolism, using ketamine as a probe. Ketamine undergoes extensive hepatic biotransformation and has been previously used to characterize the effects of imidazole anesthetics on human P-450-catalyzed drug metabolism. Ketamine N-demethylation by microsomes from three human livers was measured by gas chromatography-mass spectrometry with selected-ion monitoring. DMD was a potent, competitive inhibitor of S(+) ketamine N-demethylation, with a Kᵢ of 0.11–0.18 μM for the high affinity ketamine demethylase. The IC₅₀ for MDM inhibition of therapeutic concentrations of racemic ketamine (10 μM) was 0.15 ± 0.02 μM. Preincubation of MDM with microsomes and an NADPH generating system prior to ketamine addition had no additional effect on the inhibition of ketamine demethylation activity, thereby implicating the parent compound rather than a MDM metabolite as the inhibitory species. LMD, although pharmacologically inactive, had a greater inhibitory effect than MDM on racemic ketamine and ketamine enantiomer demethylation at therapeutic concentrations. Spectral studies showed that MDM interacted with microsomal cytochrome P-450 to elicit a Type II binding spectrum. These results demonstrate that MDM is a potent inhibitor of cytochrome P-450 catalytic activity, inhibiting ketamine demethylation by direct binding to P-450 heme iron and competitive inhibition at the substrate binding site. MDM, compared to the mixture of isomers, has a lesser potential for drug interactions at equally effective concentrations. (Key words: Anesthetics, intravenous ketamine. Interactions: drug. Metabolism: ketamine. Symphathetic nervous system, α₂-adrenergic agonist: dexmedetomidine, medetomidine. Stereochemistry.)

**Dexmedetomidine (DMED) is a new, highly selective α₂ adrenergic agonist with potent sedative, hypnotic, analgesic, sympatholytic, and anesthetic effects that is currently undergoing clinical trials.** Due to its affinity for the α₂-adrenergic receptor, DMED diminishes perioperative anxiety, anesthetic requirements, and anesthetic recovery, without serious hemodynamic, ventilatory, or other side effects, it has been advocated as a promising preanesthetic agent or anesthetic adjuvant. Levomethetomidine (LMED), the optical isomer of dexmedetomidine, is devoid of anesthetic and hemodynamic effects. The racemic mixture of DMED and LMD is known as medetomidine. The sedative, analgesic, anxiolytic, and cardiovascular effects of medetomidine and its suitability as a premedication have also been investigated recently in humans.

Medetomidine is a 4(5)-substituted imidazole, and the ability of substituted imidazoles to inhibit microsomal oxidative metabolism is well known. We have recently demonstrated that both DMED and LMD are potent inhibitors of human liver microsomal metabolism of alfentanil. Maze et al. have shown that DMED inhibits in vitro and in vivo adrenal steroidogenesis, reactions also catalyzed by cytochrome P-450 enzymes. The mechanism, however, by which the medetomidine isomers inhibit cytochrome P-450-catalyzed drug and hormone metabolism is unknown.

Ketamine undergoes extensive hepatic biotransformation by cytochrome P-450 enzymes and has been used previously to characterize the inhibitory effects of imidazole anesthetic agents on drug metabolism. The purpose of this study was to investigate the mechanism by which DMED inhibits human liver microsomal cytochrome P-450 enzymes, using ketamine as a metabolic probe. The inhibitory effects of DMED and its isomer LMD were investigated using racemic ketamine and the individual ketamine enantiomers.

**Materials and Methods**

**CHEMICALS**

Medetomidine isomers (Farnos Group, Turku, Finland) were dried at 80°C before use, and solutions were prepared fresh daily. The source of all other chemicals has been described previously.

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KETAMINE METABOLISM

All experiments were approved by the Institutional Human Subjects Review Committee. The preparation of hepatic microsomes is described in the accompanying paper.\textsuperscript{17} Ketamine N-demethylation was determined in a reaction mixture containing 0.005–5 mM ketamine, 0.01–100 \mu{}M DMED or LMED, 0.5 mg microsomal protein, and 1 mM NADPH in a total 0.5 ml 0.1 M potassium phosphate buffer (pH 7.4). Reactions (37\degree{}C) were initiated by adding NADPH following a 3-min equilibration and terminated after 10 min by adding an aliquot of the incubation mixture to 1 ml 0.1 M NaOH. For preincubation experiments, NADPH was replaced by an NADPH generating system [1 mM NADP, 10 mM glucose-6-phosphate, 1 IU/ml glucose-6-phosphate dehydrogenase, and 5 mM MgCl\textsubscript{2}]. Dexametomidine and the NADPH generating system were incubated with microsomes for 0, 10, or 20 min prior to the addition of ketamine and a second aliquot of the NADPH generating system. Reactions were terminated after an additional 10 min. The analytical procedure for norketamine quantitation by gas chromatography-mass spectrometry has been described previously.\textsuperscript{17} Medetomidine isomers had no effect on the norketamine assay.

BINDING SPECTRA

Binding of DMED to microsomal cytochrome P-450 was examined by difference spectroscopy. Spectra were obtained with a Hewlett-Packard 8450 spectrophotometer at ambient temperature. Sample and reference cuvettes contained microsomal protein (1 mg/ml) in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.2% Emulgen 911. DMED (0.1–29 \mu{}M final concentration) dissolved in buffer was added to the sample cuvette and an equal volume of buffer only was added to the reference cuvette. Total dilution did not exceed 3%.

ANALYSIS

Enzyme kinetic data were analyzed by iteratively reweighted (inverse square of the predicted velocity) nonlinear least squares regression (SPSS/PC+) of norketamine formation vs. ketamine concentration.\textsuperscript{18} The regression model chosen (number of enzymes and mode of inhibition) were based on prior graphic Lineweaver-Burke and Eadie-Hofstee analysis of the data, which also provided initial parameter estimates. Sigmoidal curves generated for DMED and LMED inhibition of ketamine N-demethylation were analyzed by nonlinear regression using a four parameter logistic equation (Allfit).\textsuperscript{19} Spectral data were analyzed by nonlinear least squares regression of absorbance peak minus absorbance trough vs. DMED concentration. Results of parameter estimates (K\textsubscript{m}, K\textsubscript{i}, and IC\textsubscript{50}) are provided along with the standard error of the parameter estimate provided by the regression program. Measured rates of norketamine formation are expressed as the mean ± standard deviation. Analysis of variance was used to test for significant differences in rates of norketamine formation.

Results

Dexametomidine was found to be a potent inhibitor of microsomal ketamine N-demethylation. Initial experiments used the clinically employed racemate at substrate concentrations reflecting plasma levels achieved during ketamine anesthesia (10 \mu{}M).\textsuperscript{20–23} The concentration-dependent effects of DMED on ketamine demethylation at this clinical concentrations are shown in figure 1. The DMED concentration required to diminish metabolism by 50% (IC\textsubscript{50}) was 0.15 ± 0.02 \mu{}M.

Ketamine demethylation is catalyzed by two apparent enzymes, a high affinity-low capacity enzyme (K\textsubscript{m} 30–50 \mu{}M) and a low affinity-high capacity enzyme (K\textsubscript{m} 600–800 \mu{}M).\textsuperscript{17} At clinical ketamine concentrations (10 \mu{}M), metabolism reflects predominantly the catalytic activity of the low K\textsubscript{m} enzyme. Results of the experiment shown in figure 1 therefore provide information only on the high-affinity enzyme, but not the low-affinity enzyme, catalyzing ketamine metabolism. Furthermore, the Michaelis-Menten inhibitory constant K\textsubscript{i} is preferable to IC\textsubscript{50} in evaluating microsomal inhibitors. K\textsubscript{i}, reflecting only the affinity of the inhibitor for an enzyme, is not dependent on the substrate used to probe the interaction and can be used to predict inhibitor effects on other substrates. In contrast, IC\textsubscript{50} is a hybrid parameter influenced by the K\textsubscript{m}.

![Fig. 1. Inhibition of racemic ketamine N-demethylation by medetomidine isomers.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931817/)

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the substrate inhibited, the substrate concentration, and its \( K_m \).

To determine the mode of inhibition and inhibitory constants, the effects of DMED on the metabolism of each ketamine enantiomer were determined separately and over a substrate concentration range necessary to achieve saturation. Lineweaver-Burke double reciprocal plots showed that DMED was a competitive inhibitor of S(+) ketamine demethylation (fig. 2 Top). Since ketamine N-demethylation is catalyzed by two apparent enzymes, and the double-reciprocal plot shown does not reflect the activity of the two enzymes, the data are also presented in a Dixon plot (fig. 2 Bottom). This plot demonstrates that both enzymes catalyzing the metabolism of S(+) ketamine were inhibited in a competitive manner by DMED. The inhibitory effectiveness of DMED is shown in figure 3, which demonstrates complete inactivation of the low \( K_m \) ketamine demethylase enzyme, transforming the normally biphasic plot of S(+) ketamine biotransformation into a straight line that reflects metabolism by only one active enzyme. DMED inhibition of S(+) ketamine metabolism was competitive for both enzymes in all three livers studied. The data were fit by nonlinear regression to a two-enzyme model in which both enzymes were inhibited competitively. The \( K_i \) for DMED inhibition of S(+) ketamine metabolism ranged from 0.11 to 0.18 \( \mu \)M for the low \( K_m \) enzyme and 0.8 to 1.4 \( \mu \)M for the high \( K_m \) enzyme (table 1). Values of \( K_m \) and \( V_{max} \) for ketamine demethylation itself were similar to those reported previously. DMED inhibition of racemic and R(−) ketamine metabolism was also competitive (not shown), similar to DMED effects on S(+) ketamine metabolism.

To examine the possibility that a metabolite of DMED was responsible for inhibition of ketamine metabolism, microsomes were preincubated with DMED and an NADPH generating system prior to addition of the substrate (fig. 4). Preincubation of DMED with microsomes had no significant effect on the rate of norketamine formation. If generation of an active DMED metabolite was required for inhibition of ketamine metabolism, a decrease in norketamine formation as a function of preincubation time would have been observed.

The effects of DMED and LMED were compared using racemic ketamine at therapeutic concentrations. LMED was no less effective as an inhibitor of ketamine metabolism than was DMED (fig. 1). The IC\(_{50}\) for LMED at 10

\[ \text{IC}_{50} \] Racemic ketamine, although the form of the compound used clinically, is metabolically and kinetically complex. It is actually two substrates, S(+) and R(−) ketamine, each of which alters significantly the metabolism of the other. Studies with racemic ketamine would yield hybrid kinetic parameters, reflecting the interaction between ketamine enantiomers as well as that between DMED and ketamine. A single enantiomer, S(+) ketamine, was therefore used as the probe substrate in experiments designed to obtain Michaelis-Menten parameters.

\[ \mu \text{M ketamine (0.14 ± 0.03 \( \mu \)M) was similar to that for DMED (0.15 ± 0.02 \( \mu \)M), although LMED appeared slightly more effective at low inhibitor concentrations.} \]

Medetomidine isomer effects on the metabolism of individual ketamine enantiomers and racemic ketamine were also investigated using a single substrate concentration (fig. 5). Both medetomidine isomers inhibited the demethylation of both S(+) and R(−) ketamine, findings replicated using all three livers. These experiments confirmed the above observation (fig. 1) that LMED was slightly more effective than DMED as an inhibitor of S(+), R(−) and racemic ketamine metabolism at low inhibitor concentrations.
MEDETOMIDINE ISOMER INHIBITION OF DRUG METABOLISM

Fig. 3. Eadie-Hofstee plot of DMED effects on S(+) ketamine N-demethylation. DMED concentrations were 0 (open circle), 0.1 (filled circle), 0.2 (open triangle), 0.4 (filled triangle), 1 (open square), or 2 (filled square) μM. Symbols denote the mean of duplicate determinations. Lines are predicted values, based on nonlinear regression analysis using a 2-enzyme model. Michaelis-Menten kinetic parameters are provided in Table I. Results are shown for HIL124.

DMED interactions with microsomal cytochrome P-450 were investigated by difference spectroscopy. Dexametomidine caused a typical Type II P-450 difference spectrum, with an absorbance maximum at 430–432 nm and a minimum at 406–408 nm (fig. 6). These spectral changes were saturable, and a Scatchard plot (fig. 7) was biphasic, indicating DMED binding to two enzymes. The following DMED spectral binding constants were obtained using microsomes from two livers: $K_a$ 0.30 ± 0.10 and 0.50 ± 0.07 μM; $K_s$ 4.3 ± 0.4 and 3.6 ± 0.3 μM. The value of these spectrally determined binding constants were quite similar to the kinetically determined inhibitory constants.

Discussion

Medetomidine isomers were potent inhibitors of S(+), R(−), and racemic ketamine N-demethylation. DMED inhibition was competitive with respect to all three substrates, and both P-450 enzymes catalyzing ketamine N-demethylation were inhibited competitively. DMED interacted with microsomal cytochrome P-450, causing a Type II difference spectrum indicative of direct DMEDimidazole nitrogen coordination to the cytochrome heme iron atom.24 It is generally accepted that other substituted imidazoles also inhibit oxidative metabolism by directly and reversibly ligating ferricytochrome heme.25,26 Previous studies have shown that substituted imidazoles do not inhibit these enzymes.27,28

![Figure 4](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931817/)

**Fig. 4.** Effect of pre-incubation on DMED inhibition of ketamine N-demethylation. Dexametomidine (0.1 μM, shaded bars) was pre-incubated with microsomes and an NADPH-generating system for 0, 10, or 20 min before addition of racemic ketamine (5 μM) and a second aliquot of the NADPH-generating system. The reaction was continued for an additional 10 min and norketamine production quantified. Open bars show controls, which were incubated without DMED prior to ketamine addition. Results are shown as the mean ± SD of four determinations. Norketamine formation was significantly different in the presence of DMED at each pre-incubation time ($P < .05$).

![Figure 5](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931817/)

**Fig. 5.** Effects of medetomidine isomers on the N-demethylation of racemic ketamine and ketamine enantiomers. Norketamine formation (mean ± SD, $n = 4$) from ketamine (5 μM) is compared in incubations containing no inhibitor (open bars), 50 nM DMED (solid bars), or 50 nM LMD (crosshatched bars). *Significantly different from control ($P < .05$). **Significantly different from control and from DMED ($P < .05$).

Table 1. Kinetic Parameters for Dexametomidine Inhibition of S(+) Ketamine Demethylation by Human Liver Microsomes

<table>
<thead>
<tr>
<th>Liver</th>
<th>$K_a$ (μM)</th>
<th>$K_s$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>114</td>
<td>0.18 ± 0.04</td>
<td>1.4 ± 0.7</td>
</tr>
<tr>
<td>116</td>
<td>0.11 ± 0.02</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>124</td>
<td>0.17 ± 0.01</td>
<td>1.4 ± 0.4</td>
</tr>
</tbody>
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Parameters (±SE of the estimate) were determined by nonlinear regression analysis.
not alter the activity of microsomal NADPH-cytochrome P-450 reductase. DMED effects on ketamine N-demethylase activity are attributed to the parent compound, rather than a DMED metabolite, since preincubation of the inhibitor with microsomes and an NADPH generating system had no effect on the degree of inhibition. Together these results suggest that DMED inhibits ketamine demethylation by ligand binding to the heme iron of cytochrome P-450 and competitive inhibition at the substrate binding site.

The potency of DMED as an inhibitor of ketamine N-demethylase is relatively unusual among 4(5)-substituted imidazoles. It is generally recognized that N-substitution confers the greatest potency among substituted imidazole inhibitors, with $K_i$ and $IC_{50}$ values in the submicromolar range. The low $K_i$ values for DMED (0.1–0.2 $\mu$M) are more characteristic of N-substituted, rather than 4(5)-substituted, imidazoles. At equivalent substrate concentrations, the $IC_{50}$ for DMED inhibition of ketamine demethylation (0.15 $\mu$M) is 20 to 30-fold less than that for inhibition by etomidate, an N-substituted imidazole which suppresses adrenal steroid metabolism and inhibits hepatic drug oxidation.

Cytochrome P-450 is actually a family of drug metabolizing enzymes, with distinct but overlapping substrate specificities. To date, at least 14 different isoforms of human P-450 have been identified. The question arises, therefore, whether DMED is a selective or nonselective inhibitor of human P-450 isoforms. Existing data suggest that DMED is nonselective, inhibiting the catalytic activity of several P-450 isoforms. Both the high- and low-affinity forms of ketamine N-demethylase were inhibited by DMED. We have also used the metabolism of the anticoagulant warfarin to probe for DMED effects on specific P-450 isoforms. DMED was a potent inhibitor of human liver microsomal warfarin (S)-7- and (S)-6-hydroxylation (P-450 2C9) and (R)-10- and (S)-4'-hydroxylation (P-450 3A4), and to a lesser extent, (R)-4'-hydroxylation (P-450 1A2). Pelkonen et al. have also shown that DMED and LMED inhibit the metabolism of probe substrates catalyzed by members of the P-450 1A, P-450 2A, P-450 2C, and P-450 3A families. Thus medetomidine isomers inhibit the activity of a broad array of cytochrome P-450 isoforms.

![Fig. 6. Difference spectra from the interaction of DMED with microsomes from one representative human liver (HL116). Each cuvette contained 1 mg/ml microsomal protein in 0.1 M potassium phosphate buffer (pH 7.40) with 0.2% Emulgen 911. DMED (0.1–29 $\mu$M final concentration) dissolved in buffer was added to the sample cuvette and an equal volume of buffer only was added to the reference cuvette. Shown are representative spectra following the addition of 0.1, 0.4, 0.7, 1.4, 4, 7, and 29 (a–g, respectively) $\mu$M DMED.](image)

![Fig. 7. Scatchard plot of the titration data excerpted in figure 6. The absorbance change ($\Delta$ABS) was calculated as the difference between absorbance maxima (430–432 nm) and absorbance minima (406–408 nm). Spectral dissociation constants, determined by nonlinear regression analysis of $\Delta$ABS versus DMED, were 0.30 ± 0.10 $\mu$M and 4.3 ± 0.4 $\mu$M.](image)
Small but significant differences in the inhibitory effects of DMED and LMD were observed in the present and previous investigations. Enantiomeric differences in inhibition of drug metabolism have been appreciated for other chiral imidazoles. For example, etomidate inhibition of adrenal steroid hydroxylase activity occurs exclusively with the d-isomer. Stereoisomers of the azole antifungal agents diniconazole and triadimeno also differ in their inhibitory potency and the spectral changes produced upon interaction with cytochrome P-450. It is perilous to predict, based exclusively on in vitro data, whether a clinical drug interaction will occur. A significant drug interaction might be expected at inhibitor concentrations one tenth of the \( K_i \). This would require intrahepatic DMED concentrations of approximately 10–20 nM. In rats, DMED (80 \( \mu \text{g/kg} \)) administration resulted in peak intrahepatic concentrations of approximately 5,000 nM, despite peak plasma concentrations of only 100–200 nM. LMD caused a significant prolongation of hexobarbital sleeping times, due to a pharmacokinetic interaction, albeit only at higher doses. In dogs, DMED (80 \( \mu \text{g/kg} \)) has been shown to diminish canine adrenal steroidogenesis in vivo, and medetomidine (40–80 \( \mu \text{g/kg} \)) has been associated with a prolonged duration of anesthesia and recovery from ketamine. Thus medetomidine isomers can inhibit the metabolism of endogenous and exogenous cytochrome P-450 substrates in animals. Investigations are clearly necessary to assess the effects of DMED on drug and hormone disposition in humans where peak plasma concentrations are approximately 50 nM and intrahepatic concentrations have not yet been established.

Differences in the inhibitory activity of DMED and LMD, although statistically significant, were small and not likely of clinical significance. What clearly is of potential clinical importance however, is that both isomers inhibit cytochrome P-450 activity whereas only DMED possesses anesthetic and analgesic efficacy. Racemic medetomidine is thus less potent therapeutically while possessing a greater potential for drug interactions. These data support the further clinical development of DMED in preference to the racemate.

In summary, we have shown that DMED is a potent competitive inhibitor of human liver ketamine N-demethylation, interacting directly with the heme iron atom of microsomal cytochrome P-450. DMED is a relatively nonspecific inhibitor, diminishing the activity of several different P-450 isozymes. LMD, the optical isomer of DMED, is at least an equally effective inhibitor while lacking anesthetic-analgesic activity.

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References


