LABORATORY REPORT

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Influence of Dexmedetomidine and Clonidine on Human Liver Microsomal Alfentanil Metabolism

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Perioperative administration of the α₂ agonist clonidine has been shown to increase plasma alfentanil concentrations; however, the mechanism for this pharmacokinetic drug interaction is unknown. Because alfentanil undergoes extensive hepatic biotransformation, clonidine inhibition of alfentanil metabolism may alter alfentanil disposition. The first purpose of this investigation was to test the hypothesis that clonidine impairs human liver alfentanil metabolism. The new highly selective α₂ agonist dexmedetomidine (D-metedomidine) is a substituted imidazole and thus may inhibit hepatic drug biotransformation. The second purpose of this study, therefore, was to assess the effect of D-metedomidine and its levorotatory (L) isomer on alfentanil biotransformation. Human liver microsomal alfentanil metabolism was assessed in vitro using a gas chromatography–mass spectrometry assay. Clonidine, at concentrations as great as 10 μM (far exceeding therapeutic levels), had no significant effect on alfentanil oxidation. In contrast, D-metedomidine and its optical isomer L-metedomidine were potent inhibitors of human liver microsomal alfentanil metabolism. The concentration producing 50% inhibition (IC₅₀) of alfentanil (10 μM) oxidation was 0.7–1.0 and 2.8–4.0 μM for D-metedomidine and L-metedomidine, respectively. Preincubation of D-metedomidine with microsomes did not enhance the inhibition of alfentanil metabolism. These results suggest that the increased alfentanil plasma concentrations and potentiation of alfentanil anesthesia associated with clonidine do not result from clonidine inhibition of alfentanil metabolism. D-metedomidine impairment of alfentanil metabolism, however, if present at therapeutic concentrations, may influence alfentanil disposition. (Key words: Anesthetics, intravenous: alfentanil. Interactions: drug. Metabolism: alfentanil. Sympathetic nervous system, α₂-adrenergic agonist: dexmedetomidine; clonidine.)

α₂-ADRENERGIC AGONISTS are currently under investigation as novel anesthetic agents because they produce sedation, anxiolysis, and analgesia and reduce heart rate and blood pressure without causing respiratory deprevation.¹ Preanesthetic administration of clonidine improves perioperative hemodynamic stability and markedly decreases the intraoperative requirement for volatile anesthetics² and opioids such as fentanyl and sufentanil.³–⁵ Segal et al. have recently shown that perioperative clonidine administration resulted in a 1.5-fold increase in intraoperative plasma alfentanil concentrations, suggesting a pharmacokinetic component to the mechanism for clonidine potentiation of alfentanil analgesia.⁶ One hypothesis proposed to explain this kinetic interaction is clonidine inhibition of cytochrome P-450 and impairment of alfentanil metabolism.⁷ Alfentanil is eliminated exclusively by hepatic metabolism,⁸ and inhibition of alfentanil metabolism may substantially reduce plasma alfentanil clearance, thereby prolonging the effect.⁷,⁸ One purpose of this investigation, therefore, was to determine the influence of clonidine on human liver microsomal metabolism of alfentanil.

Medetomidine is a new, highly selective α₂ agonist with potent anesthetic and analgesic properties.⁹ Medetomidine exists as an equal mixture of two stereoisomers (i.e., as a racemate). The sedative, hemodynamic, and endocrine effects of the racemic mixture in humans have been described.¹⁰,¹¹ Efficacy has been attributed exclusively to the dextro (D) isomer, dexmedetomidine.⁹,¹² D-metedomidine alone has been shown in animals and in humans to possess the sedative, analgesic and hemodynamic properties of the racemic mixture. D-metedomidine is currently under investigation as a preanesthetic agent.¹³,¹⁴ Medetomidine is a 4(5)-substituted imidazole, and the ability of certain substituted imidazoles to inhibit microsomal oxidative metabolism is well known.¹⁵ Therefore, the second purpose of this investigation was to examine the influence of D- and L-metedomidine isomers on microsomal alfentanil biotransformation.

Materials and Methods

Alfentanil and R-38-527 (the alfentanil assay internal standard) were obtained from the Janssen Research Foundation (Piscataway, NJ) and Beerse, Belgium. Metedomidine isomers were obtained from Farmos Group Ltd. (Turku, Finland). All other reagents were from Sigma (St. Louis, MO).

Human livers were obtained from organ transplant donors and stored at −80°C until used. Microsomes were
prepared as described previously. Microsomal protein concentrations were determined by the method of Lowry et al., using bovine serum albumin as the standard. Microsomal cytochrome P-450 content was 0.4–0.5 nmol/mg protein.

Reaction mixtures (37°C, 10 min) contained 0.5 mg microsomal protein, 10–25 μM alfentanil, 0.01–100 μM inhibitor (clonidine or D- or L-metomidine), and 1 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH) in a total volume of 0.5 ml 100 mM potassium phosphate buffer (pH 7.4). Reactions were initiated by adding NADPH and terminated by adding 0.5 ml 0.1 M sodium hydroxide. For time-course experiments, reaction volume was increased to 2.0 ml, and 0.25-ml aliquots were removed at intervals and added to an equal volume of sodium hydroxide. For preincubation experiments, NADPH was replaced by an NADPH-generating system (1 mM nicotinamide adenine dinucleotide phosphate [NADP], 10 mM glucose-6-phosphate, 1 IU/ml glucose-6-phosphate dehydrogenase, and 5 mM magnesium chloride). D-metomidine and the NADPH-generating system were incubated with microsomes for 15 min before addition of 10 μM alfentanil and an additional aliquot of the NADPH-generating system. Reactions were terminated after an additional 10 min. Total alfentanil metabolism was determined from the disappearance of substrate.

Microsomal alfentanil concentrations were measured by a modification of the method of Woestenborghs et al. After addition of the internal standard R-38-527 (N-[1-[3-(4-ethyl-4,5-dihydro-5-oxo-1H-tetrazol-1-yl)propyl]-4-(methoxymethyl)-4-piperidinyl]-N-phenylpropanamide), the basic mixture was twice extracted with 4 ml heptane: isoamyl alcohol (98.5:1.5). The combined organic layers were evaporated to dryness under nitrogen and reconstituted in 50 μl methanol, and 1–5 μl was injected into the gas chromatograph. Extraction recoveries averaged 94% using this protocol. A Hewlett Packard 5890/5970 gas chromatograph–mass selective detector containing a DB-1701 (10 m × 0.18 mm × 0.4 μm) capillary column was used. Injector (splitless), oven, and transfer line temperatures were 290, 150–280 at 25°/min, and 280°C, respectively. Helium was used as the carrier gas. Alfentanil and the internal standard were detected by selected ion monitoring at mass/charge ratios of 289 (alfentanil) and 282 (R-38-527). Standard curves of peak area ratios (alfentanil/internal standard) versus nanomoles alfentanil added were prepared and used to quantify alfentanil in unknowns. The α agonists had no effect on the recovery or quantification of microsomal alfentanil.

Alfentanil disappearance data were analyzed by log linear regression. Concentration–effect data for metomidine inhibition of alfentanil oxidation were analyzed by nonweighted nonlinear regression using a four-parameter logistic equation (Allfit). Effects of clonidine and of D-metomidine preincubation on alfentanil metabolism were assessed by analysis of variance. All results are expressed as the mean ± standard error.

Results

Alfentanil was rapidly metabolized by human liver microsomes (fig. 1). The disappearance of alfentanil exhibited first-order kinetics, both in the absence and presence of metomidine isomers. The rate constant for alfentanil disappearance, determined by linear regression analysis of the log concentration versus time curve, was 0.065 min⁻¹. Both D- and L-metomidine inhibited the disappearance of alfentanil from the microsomal preparation (fig. 1). D-metomidine had a greater inhibitory effect than L-metomidine at 2 μM (k = 0.011 vs. 0.039 min⁻¹). This was confirmed in concentration–effect experiments (fig. 2). D-metomidine inhibited alfentanil metabolism over the range 0.1–100 μM. The D-metomidine concentration producing 50% inhibition of alfentanil (10 μM) oxidation (IC₅₀) was 0.7 and 1.0 μM in microsomes from two different livers. The optical isomer L-metomidine was approximately 5-fold less potent: its IC₅₀s were 2.8 and 4.0 μM (fig. 2). Similar results (not shown) were obtained using 25 μM alfentanil (IC₅₀ 0.4 and 5.7 μM for D- and L-metomidine, respectively).

To examine the possibility that a metabolite of D-metomidine was responsible for inhibition of alfentanil me-

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**FIG. 1.** Semilogarithmic plot of alfentanil metabolism by human liver microsomes (liver 115). Alfentanil concentration was 10 μM, and microsomes were present at 1 mg/ml. At the indicated time points, aliquots were removed for determination of alfentanil concentration. Incubations also contained 2 μM D-metomidine (squares), 2 μM L-metomidine (triangles), or no other drug (controls; circles). Data points are the mean ± SEM of four determinations.
Fig. 2. Inhibition of alfentanil (10 μM) oxidation by medetomidine isomers. Results are the mean of duplicate incubations. Left: Microsomes from liver 116. The IC_{50}s were 1.0 and 4.0 μM for d- and l-medetomidine, respectively. Right: Microsomes from liver 115. The IC_{50}s were 0.7 and 2.8 μM for d-medetomidine and l-medetomidine, respectively.

Fig. 3. Effect of preincubation on d-medetomidine inhibition of alfentanil oxidation. d-medetomidine (2 μM, shaded bars) was preincubated with microsomes and an NADPH-generating system for 0 or 15 min prior to addition of alfentanil (10 μM). The reaction was continued for an additional 10 min, and alfentanil oxidation was then determined. Open bars show controls, which were incubated for 0 or 15 min without dmedetomidine prior to alfentanil addition. Results are shown as the mean ± SEM of eight determinations.

Tab. 1. Effect of Clonidine on Alfentanil Metabolism

<table>
<thead>
<tr>
<th>Clonidine (μM)</th>
<th>Alfentanil Metabolism (nmol/10 min)</th>
<th>Liver 115</th>
<th>Liver 116</th>
<th>Liver 118</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>2.79 ± 0.13</td>
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<tr>
<td>0.001</td>
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<tr>
<td>0.01</td>
<td>3.16 ± 0.11</td>
<td>4.13 ± 0.12</td>
<td>2.18 ± 0.06</td>
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<tr>
<td>1</td>
<td>2.92 ± 0.15</td>
<td>4.29 ± 0.03</td>
<td>2.12 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.61 ± 0.27</td>
<td>4.18 ± 0.12</td>
<td>1.91 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>2.41 ± 0.06</td>
<td>4.06 ± 0.02</td>
<td>1.52 ± 0.12*</td>
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</table>

Reaction mixtures contained 0.5 mg protein, 10 μM alfentanil, and 1 mM NADPH in 0.5 ml 0.1 M phosphate buffer. Alfentanil metabolism was quantified as described in Materials and Methods. Results are the mean ± SEM of three determinations.

* Significantly different from control (P < 0.05).

Discussion

Alfentanil undergoes extensive biotransformation: less than 1% is recovered intact in human urine. The primary route of metabolism is N-dealkylation at the piperidine nitrogen to form noralfentanil. Microsomal alfentanil metabolism also results in the formation of N-phenylpropanamide via oxidative N-dealkylation at the amide nitrogen. Assays of alfentanil metabolism by measurement of noralfentanil formation or by measurement of alfentanil disappearance produce similar results at substrate concentrations identical to those used in the current experiments. Rates of apparent alfentanil oxidation determined in the current investigation are similar to those reported previously.

At plasma concentrations of 5–7 nM, clonidine has been shown to produce a 1.5-fold increase in intraoperative
alfentanil plasma concentrations. Clonidine has been shown also to alter the disposition of other drugs undergoing oxidative dealkylation. Clonidine inhibits lidocaine deethylation in mice and rats, resulting in increased plasma lidocaine concentrations. In the current investigation, microsomal alfentanil oxidation was altered only negligibly by clonidine concentrations as high as 100 μM. These results suggest that the increased alfentanil plasma concentration and potentiation of alfentanil anesthesia in patients receiving clonidine are clearly not the result of clonidine inhibition of alfentanil metabolism. Furthermore, inhibition of alfentanil metabolism is not a general property of α2 agonists.

In contrast to the results with clonidine, we found that the medetomidine isomers were potent inhibitors of human microsomal alfentanil oxidation. The average IC50 for inhibition of alfentanil oxidation was 0.8 and 3.2 μM for 1- and 1-medetomidine, respectively. This IC50 value for 1-medetomidine was similar to the inhibition constant (K) and IC50 values for 1-medetomidine inhibition of human microsomal ketamine N-demethylation. Preincubation of microsomes with 1-medetomidine did not enhance the inhibition of alfentanil metabolism, indicating that 1-medetomidine, rather than a metabolite, was responsible for the inhibitory effects of this α2 agonist on alfentanil oxidation.

There exists an extensive literature describing the ability of substituted imidazoles to inhibit microsomal oxidative metabolism. For example, 4-substituted imidazole H2-antagonists and N(1)-substituted imidazole antimycotic agents are among the most potent inhibitors of cytochrome-P-450-mediated drug and steroid oxidations. Substituted imidazoles exhibit a clear structural specificity for P-450 inhibition. For example, the potency of 1- and 4(5)-substituted imidazoles is approximately 100 times greater than that of 2-substituted imidazoles. In general, N(1)-substituted imidazoles are the most potent inhibitors in this drug class. However, we found that the IC50 of the 4(5)-substituted 1-medetomidine was equivalent to that reported for the most potent N-substituted antimycotic agents. Thus, 1-medetomidine is a potent inhibitor of oxidative drug metabolism and is a novel 4(5)-substituted imidazole because of its high potency.

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