Pharmacokinetics of remifentanil in conscious cats and cats anesthetized with isoflurane

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Objective—To characterize the pharmacokinetics of remifentanil in conscious cats and cats anesthetized with isoflurane.

Animals—6 cats.

Procedures—Remifentanil (1 µg/kg/min for 5 minutes) was administered IV in conscious cats or cats anesthetized with 1.63% isoflurane in oxygen in a randomized crossover design. Blood samples were obtained immediately prior to remifentanil administration and every minute for 10 minutes, every 2 minutes for 10 minutes, and every 5 minutes for 10 minutes after the beginning of the infusion. Blood was immediately transferred to tubes containing citric acid, flash frozen in liquid nitrogen, and stored at –80°C until analysis. Blood remifentanil concentration was determined by use of liquid chromatography–mass spectrometry. Remifentanil concentration-time data were fitted to compartment models.

Results—A 2-compartment model (with zero-order input because of study design) best described the disposition of remifentanil in awake and isoflurane-anesthetized cats. The apparent volume of distribution of the central compartment, the apparent volume of distribution at steady state, the clearance, and the terminal half-life (median [range]) were 1.596 (1.164 to 2.111) and 567 (278 to 641) mL/kg, 7632 (2,284 to 76,039) and 1,651 (446 to 29,229) mL/kg, 766 (408 to 1,473) and 371 (197 to 472) mL/min/kg, and 17.4 (5.5 to 920.3) and 15.7 (3.8 to 410.3) minutes in conscious and anesthetized cats, respectively.

Conclusions and Clinical Relevance—The disposition of remifentanil in cats was characterized by a high clearance. Isoflurane anesthesia significantly decreased the volume of the central compartment, likely by decreasing blood flow to vessel-rich organs. (Am J Vet Res 2008;69:531–536)

Remifentanil is a synthetic opioid in the phenylpiperidine series.1 It is a full agonist of µ-opioid receptors.2 In dogs and humans, remifentanil does not undergo hepatic metabolism, but it is degraded by non-specific plasma and tissue esterases, a unique feature among opioids.1,3 Remifentanil is therefore predicted to have a high clearance, independent of organ function. This was confirmed in humans because severe renal or liver disease does not influence the pharmacokinetics of remifentanil.3,4 An opioid with such characteristics would be useful in anesthesia of cats, for patients with liver or renal disease, as well as for balanced anesthesia, particularly for long procedures. Effects of drugs with a high clearance are expected to be less prolonged after long infusions than those of drugs with a lower clearance.3

Cats have been reported to be deficient in some pathways used in the metabolism of xenobiotics.3 This may lead to prolonged duration of action, increased adverse effects, or increased risk of toxicosis related to some drugs. Therefore, drugs not relying on hepatic biotransformation for their elimination are of particular interest in cats. Cats have also been reported to have lower cholinesterase and pseudocholinesterase activities than humans.7–9 Compared with dogs, results of 3 studies7–9 suggest that cats have similar or slightly lower cholinesterase and pseudocholinesterase activities. However, similar data for other types of plasma or tissue esterases have not been published. It is nevertheless possible that the clearance of remifentanil is different in cats than in dogs or humans.

Various factors have been reported to influence the concentration of cholinesterase and pseudocholinesterase in cat plasma and CSF.10–12 In that study, central depressants decreased CSF cholinesterase activity. Moreover, inhalant anesthetics influence the disposition of other drugs in cats, likely by decreasing blood flow to excretory organs.11 Inhalant anesthesia could therefore affect the disposition of remifentanil both directly, by affecting esterase activity, and indirectly, by decreasing

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>LC-MS</td>
<td>Liquid chromatography–mass spectrometry</td>
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<tr>
<td>AUC</td>
<td>Area under the concentration-time curve</td>
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<tr>
<td>AUC∞</td>
<td>Area under the blood concentration-time curve extrapolated to infinity</td>
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<td>$C_{max}$</td>
<td>Maximum blood concentration</td>
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blood flow to tissues able to metabolize this drug. It is nevertheless expected that the disposition of remifentanil in conscious cats would be less affected by anesthesia than the disposition of drugs requiring organ metabolism.

The purpose of the study reported here was to characterize the disposition of remifentanil in conscious cats and in cats anesthetized with isoflurane. We hypothesized that remifentanil would be rapidly cleared from blood and that anesthesia with isoflurane would have minimal effect on its pharmacokinetics.

**Materials and Methods**

Six adult healthy female cats (mean ± SD weight, 4.2 ± 0.4 kg) were used. The study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

For studies in conscious cats, the day prior to an experiment, cats were anesthetized by use of isoflurane in oxygen. A 22-gauge, 10-cm catheter was placed in a jugular vein for blood sampling. A 22-gauge, 2.5-cm catheter was placed in a medial saphenous vein for drug administration. A light bandage was placed over the catheters, and cats were allowed to recover from anesthesia. For studies in anesthetized cats, anesthesia was induced in an acrylic chamber by use of isoflurane in oxygen. After endotracheal intubation, anesthesia was maintained with isoflurane in oxygen delivered via a coaxial Mapleson F circuit. Oxygen flow was 200 mL/kg/min. Venous catheters were placed in a similar manner to studies in conscious cats. Lactated Ringer’s solution was administered at 3 mL/kg/h via the medial saphenous catheter. Body temperature was continuously measured with a temperature probe, calibrated before each study against a certified thermometer, and positioned in the distal third of the esophagus. Body temperature was maintained between 37° and 38°C by supplying external heat as needed. Inspired and end-tidal oxygen and carbon dioxide partial pressures and isoflurane concentrations were measured continuously by use of a Raman spectrometer. In addition, end-tidal isoflurane concentration was measured every 15 minutes from samples collected by hand, by use of an infrared analyzer. This analyzer was calibrated daily against room air and standards of known isoflurane concentration (0.5%, 1.5%, and 2.5%). End-tidal isoflurane concentration was set at 1.63% and maintained at that concentration for the remainder of the study. Sixty minutes were allowed for conditions to equilibrate before remifentanil administration.

The day after instrumentation for studies in conscious cats, or after 60 minutes of stable isoflurane anesthesia for studies in anesthetized cats, remifentanil (1 µg/kg/min for 5 minutes) was administered IV, via the medial saphenous catheter. The order of the studies was selected randomly, and at least 2 weeks were allowed between studies. Blood samples (1 mL) were collected from the jugular catheter immediately prior to remifentanil administration and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, and 30 minutes after the beginning of the infusion. Blood samples were immediately transferred to tubes containing 30% citric acid for prevention of remifentanil hydrolysis via pH control, flash frozen in liquid nitrogen, and stored at −80°C until analysis.

Remifentanil concentration was measured in feline blood via LC-MS by use of a modification of the methods reported by Bender et al. Acetic acid, acetonitrile, and water were high-performance liquid chromatography grade, and formic acid was spectrophotometric grade. An analytic reference standard of fentanyl was used.

Remifentanil was quantitated in feline blood via LC-MS analysis of extracted blood samples. The calibration standards were prepared with stock solutions made by dissolving 10.0 mg of remifentanil standard in 10.0 mL of acetonitrile. Working solutions were prepared by dilution of the remifentanil stock solution with acetonitrile to concentrations of 100 and 500 ng/mL. Blood calibrators were prepared by dilution of the working remifentanil solutions with drug-free blood to concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality-control samples (blood fortified with analytes at concentrations midway of the standard curve) were routinely included as an additional check of accuracy. The concentration of remifentanil in each sample was determined by use of the internal standard method via peak area ratio and linear regression analysis. Fentanyl was used as internal standard.

The limit of quantification was 0.05 ng/mL.

Quantitative analyses were performed on a mass spectrometer coupled with a liquid chromatography system. Chromatography was performed with a 3-cm × 2.1-mm, 3-µm column and a linear gradient of acetonitrile in water with a constant 0.2% formic acid at a flow rate of 0.4 mL/min. The acetonitrile concentration was held at 10% for 0.4 minutes, then increased to 90% over 7 minutes. Prior to analysis, blood samples, standards, and quality-control samples were allowed to thaw at 20°C. The plasma proteins were extracted by precipitation with the addition of 0.5 mL of acetonitrile containing 10 ng of internal standard/mL. All samples were vortex mixed for 1.5 minutes, followed by centrifugation (2,000 × g for 5 minutes at 4°C). The injection volumes were 10.0 µL.

Detection and quantification was performed via full scan LC-MS transitions of initial precursor ion for remifentanil (mass-to-charge ratio, 377). The response for the major product ions for remifentanil (mass-to-charge ratio, 317 and 285) was plotted, and peaks at the proper retention time were integrated by use of a computer program. This program was used to generate calibration curves and quantitate these analytes in all samples.

For the evaluation of the accuracy and precision of the method, quality-control samples were prepared by spiking blank blood samples with remifentanil to concentrations of 0.1, 1.0, and 10 ng/mL. The standards and the quality-control samples were treated in the same way as the samples. The method was linear between 0.05 and 50 ng/mL (R² ≥ 0.998). Interassay and intra-assay coefficients of variation were < 10%.

The AUC was assessed via the linear trapezoidal method. Nonlinear least squares regression was performed on blood remifentanil concentrations following IV administration of a short infusion with the use of a computer software program. Data were weighted by the reciprocal of the predicted blood remifentanil concentration squared. Data from each cat were fitted to 1-, 2-, and
3-compartment models with zero-order input. The appropriate model was selected by observation of the residual plot and by use of the Akaike information criterion. Standard compartmental equations were used to estimate pharmacokinetic parameters for each cat. Because raw pharmacokinetic data were not normally distributed, they were log-transformed prior to analysis. Normal distribution of the log-transformed pharmacokinetics parameters was verified by use of the Shapiro-Wilk test. Log-transformed parameters in conscious and anesthetized cats were compared by use of a 2-tailed paired t test. Differences were considered significant at P < 0.05. Data are presented as median (range).

Results

A 2-compartment model best described the decrease in blood remifentanil concentration following a short IV infusion in conscious and isoflurane-anesthetized cats (Figure 1). In conscious and anesthetized cats, respectively, the apparent volume of distribution of the central compartment, the apparent volume of distribution at steady state, the clearance, and the terminal half-life were 1.596 (1.164 to 2.111) and 567 (278 to 641) ml/kg, 7.632 (2.284 to 76.039) and 1,651 (446 to 29.229) ml/kg, 766 (408 to 1,473), and 371 (197 to 472) ml/min/kg, and 17.4 (5.5 to 920.3) and 15.7 (3.8 to 410.3) minutes. The apparent volume of distribution of the central compartment and clearance were significantly (P < 0.001) smaller in cats anesthetized with isoflurane than in conscious cats. The AUC∞ and Cmax were 6.3 (3.4 to 12.3) and 13.5 (10.6 to 25.4) ng•min/ml and 1.1 (0.8 to 2.2) and 4.0 (2.7 to 5.1) ng/ml in conscious and anesthetized cats, respectively. The AUC∞ and Cmax were significantly (P < 0.001) greater in cats anesthetized with isoflurane than in conscious cats. Pharmacokinetic parameters for remifentanil in conscious and isoflurane-anesthetized cats were summarized (Table 1).

Discussion

The disposition of remifentanil was characterized by a moderate volume of distribution and a high clearance, which resulted in a short terminal half-life. Isoflurane anesthesia decreased both volume of distribution and clearance, which resulted in no significant change in terminal half-life. In the present study, remifentanil concentrations were determined in blood after a short IV infusion and a 30-minute sampling duration. Pharmacokinetic studies commonly use plasma or serum rather than blood concentrations. However, for drugs such as remifentanil, which are metabolized in blood and plasma, it has been recommended to use blood to avoid the risks of degra-

Table 1—Pharmacokinetic parameters (median [range]) for remifentanil following IV administration of an infusion (1 µg/kg/min for 5 minutes) in conscious and isoflurane-anesthetized cats (n = 6).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conscious</th>
<th>Anesthetized</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>A (ng/mL)</td>
<td>3.0 (2.2–4.1)</td>
<td>8.8 (7.7–17.1)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>B (ng/mL)</td>
<td>0.13 (0.06–0.28)</td>
<td>0.13 (0.05–0.91)</td>
<td>NS</td>
</tr>
<tr>
<td>α (min)</td>
<td>0.05 (0.001–0.13)</td>
<td>0.05 (0.002–0.18)</td>
<td>NS</td>
</tr>
<tr>
<td>β (min)</td>
<td>17.4 (5.6–920.3)</td>
<td>15.7 (3.8–410.3)</td>
<td>NS</td>
</tr>
<tr>
<td>k(1) (l/min)</td>
<td>0.29 (0.05–0.67)</td>
<td>0.63 (0.14–0.80)</td>
<td>NS</td>
</tr>
<tr>
<td>k(2) (l/min)</td>
<td>0.20 (0.09–0.78)</td>
<td>0.14 (0.11–0.17)</td>
<td>NS</td>
</tr>
<tr>
<td>k(3) (l/min)</td>
<td>0.08 (0.01–0.17)</td>
<td>0.06 (0.004–0.22)</td>
<td>NS</td>
</tr>
<tr>
<td>V(1) (ml/kg)</td>
<td>1.596 (1.164–2.111)</td>
<td>567 (278–841)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>V(2) (ml/kg)</td>
<td>5.671 (1.118–7.713)</td>
<td>1.094 (168–28.588)</td>
<td>NS</td>
</tr>
<tr>
<td>V(3) (ml/kg)</td>
<td>7.632 (2.284–76.039)</td>
<td>1.651 (446–29.229)</td>
<td>NS</td>
</tr>
<tr>
<td>Clearance (l/min/kg)</td>
<td>766 (408–1,473)</td>
<td>371 (197–472)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AUC∞ (ng•min/ml)</td>
<td>6.1 (2.8–11.5)</td>
<td>12.8 (10.3–24.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AUC∞ (ng•min/ml)</td>
<td>6.5 (3.4–12.3)</td>
<td>13.5 (10.6–25.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Vp (l/min)</td>
<td>2.0 (0.6–22.9)</td>
<td>2.6 (1.4–13.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>V∞ (ml/kg)</td>
<td>5.1 (2.5–5)</td>
<td>5.3 (5–5)</td>
<td>NS</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>1.0 (0.6–1.6)</td>
<td>3.2 (2.1–4.1)</td>
<td>&lt; 0.001</td>
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</table>

A value of P < 0.05 was considered significant. t1/2 = Distribution half-life, tmax = Elimination half-life, k(1), k(2), and k(3) = Microrate constants. Vp = Apparent volume of the central compartment. Vc = Apparent volume of the peripheral compartment. Vp = Apparent volume of distribution at steady state. AUC∞ = Area under the plasma concentration-time curve extrapolated. Tmax = Time to reach the maximal concentration. NS = Not significant.
dation associated with the additional time required for separation of plasma or serum from cells. Moreover, because published studies reporting the pharmacokinetics of remifentanil also used blood concentrations, comparisons with the present study are more meaningful than if plasma or serum concentrations had been used. Administration of a short infusion was selected because of concerns that bolus administration of a similar dose of remifentanil would result in dysphoria in the conscious cat group because of the higher blood and effect-site concentrations associated with bolus administration. Infusions are preferred for drugs with a narrow therapeutic index. A short infusion allows better characterization of the initial phase than a long infusion. Moreover, the administration in the present study mimicked that used in a published study in dogs, thus facilitating comparisons between the 2 studies. Duration of sampling was based on previous studies in other species, which detected rapid disappearance of remifentanil from blood. Although seemingly short, duration of sampling in the present study was adequate because, by 30 minutes, blood remifentanil concentration was equal to or less than the assay’s limit of quantification (0.05 ng/mL), except for 2 samples in which it was close to that limit (0.06 and 0.07 ng/mL). Therefore, sampling for a longer duration would not have resulted in additional information on the disposition of remifentanil. To prolong the time during which measurable concentration could be detected, the sensitivity of the assay would need to be improved. However, the methods were already modified and refined to reach a limit of quantification of 0.03 ng/mL, compared with 0.1 ng/mL in most published studies. Alternatively, a higher dose of remifentanil could have been administered. Although this may have improved the accuracy of the pharmacokinetic analysis, as mentioned, opioids may cause dysphoria in cats in a dose-dependent manner, which was a concern because of the necessity for frequent blood sampling.

The concentration-time profile of remifentanil in cats was best described by a 2-compartment model, with rapid distribution and elimination. This is in accordance with published studies in dogs and humans. Other studies in humans have revealed that the disposition of remifentanil was best described by a 3-compartment model. In the present study, a 3-compartment model did not improve objective measurements of goodness of fit such as the weighted sum of square of the residuals or the Akaike information criterion, in comparison to a 2-compartment model. The simpler model was therefore selected.

In comparison to dogs and humans, the volume of distribution of remifentanil was larger and the clearance was higher. This resulted in a longer terminal half-life in cats than in dogs or humans. Differences in volume of distribution may be related to differences in body composition. However, results of 1 study suggest that body composition in dogs and cats is similar. The detected differences are actually likely related to study design. The cited studies in dogs and humans used arterial blood samples for analysis, whereas venous blood was used in the present study. Arterial concentration is expected to be higher than venous concentration, particularly for drugs undergoing elimination in peripheral tissues. This will result in the calculation of smaller volumes of distribution when arterial concentrations are used for pharmacokinetic analysis. Arterial sampling was not used in this study because of the technical difficulties associated with insertion and maintenance of arterial catheters in cats. Moreover, it has recently been reported that for a drug such as remifentanil, which undergoes tissue elimination, venous concentrations reflect effect-site concentrations (ie, CNS concentrations) better than arterial concentrations. The higher clearance of remifentanil in cats than in dogs may be related to similar factors: because venous concentrations are expected to be lower than arterial concentrations, the AUC derived from these venous concentrations will be smaller than if derived from arterial concentrations. Clearance is calculated as the ratio of the dose and the AUC, and for a given dose, a smaller AUC will correspond to a higher clearance. Regardless of these differences, the disposition of remifentanil in cats had similar characteristics to that in humans and dogs (ie, a relatively small volume of distribution and a high clearance). Although terminal half-life in cats was longer than that reported in dogs and in most studies in humans, it was in the same order of magnitude and much shorter than the terminal half-life of fentanyl in cats (2.35 hours).

In the present study, anesthetized cats were administered 1.63% isoflurane. This concentration corresponds to the minimum alveolar concentration. It was selected to induce a moderate depth of anesthesia, under the assumption that remifentanil would somewhat reduce anesthetic requirements. Isoflurane anesthesia altered the disposition of remifentanil in cats. The volume of distribution of the central compartment and clearance were smaller and Cmax and AUC larger in isoflurane-anesthetized cats than in conscious cats. These changes are likely related to the cardiovascular effects of isoflurane, which reduces cardiac output and therefore blood flow to vessel-rich organs and tissues involved in the degradation of remifentanil. Alternatively, a reduction of cardiac output per se may result in increased Cmax and AUC because the drug would be diluted in a smaller cardiac output volume. This is the principle on which indicator dilution techniques for the measurement of cardiac output are based. Isoflurane anesthesia did not affect the distribution phase and terminal half-life was similar in conscious and anesthetized cats because of reductions in both volume of distribution and clearance in anesthetized cats.

A relatively large variability in the disposition of remifentanil was evident among individual cats. Various factors have been reported to affect pharmacokinetic variability. These include age, body weight, sex, disease, and genetics. In the present study, female cats of similar age and body weight were used. It is therefore likely that the variability detected had a genetic basis. Recent studies have revealed the large influence of genetics on drug metabolism. Moreover, it is known that in humans, genetic variability is responsible for variations in activity of plasma cholinesterase and likely other esterases as well.
Some of the extreme values reported in the present study may not be truly representative of the disposition of remifentanil in cats. For example, according to the model, remifentanil had an extremely long terminal half-life in 1 conscious cat and 1 anesthetized cat (920 and 410 minutes, respectively). The next highest values were 56 and 75 minutes in the conscious and anesthetized cats, respectively. The high values may be related to the fact that in these cats, at the later times, blood concentration was not changing, resulting in a small slope of the elimination phase. These values were close or equal to the limit of quantification of the assay, making their accuracy questionable and the assessment of further decrease in concentration impossible. This may have resulted in an underestimation of the terminal slope for some cats. Alternatively, it is possible that the terminal phase of the disposition of remifentanil is actually slow. The existence of a slow terminal phase was suggested in humans. Its clinical importance, however, was questioned because the fractional coefficient for that phase was small. In the present study, for the 2 cats having the longest terminal half-lives, fractional coefficients for the terminal phase were 0.7% and 1.5%, and that phase would therefore be expected to have a small impact on the decrease in blood remifentanil concentration after discontinuation of an infusion.

The disposition of remifentanil in cats was characterized by rapid distribution and high clearance. Anesthesia with isoflurane modestly affected the pharmacokinetics of remifentanil. It is expected that remifentanil will rapidly achieve steady state during infusion and will rapidly disappear from blood after cessation of an infusion. Pharmacokinetic parameters reported here will allow the design of adequate administration schemes and the prediction of blood concentration during infusion of remifentanil to healthy cats. Further studies are necessary to establish target blood concentrations for desired effects such as analgesia or a decrease in anesthetic requirements.

References

remifentanil (GI87084B) and its major metabolite (GI90291) in patients undergoing elective inpatient surgery. Anesthesiology 1995;79:893–903.


