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Case Study

Prolonged Anesthetic Recovery after Continuous Infusion of Midazolam in 2 Domestic Cats (*Felis catus*)

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Two healthy research cats involved in a randomized, blinded prospective pharmacodynamics study evaluating midazolam continuous-rate infusion as a means to decrease sevoflurane concentrations experienced unexpectedly prolonged recoveries. Midazolam loading doses, infusion rates, and the targeted plasma midazolam concentrations at steady-state were determined by pharmacokinetic modeling based on the results of a preliminary pharmacokinetic study using a single dose of midazolam. In the pharmacodynamics study, cats remained oversedated after recovery from anesthesia, and plasma concentrations of midazolam and its primary metabolite (1-hydroxymidazolam) remained elevated. The use of flumazenil was unsuccessful in timely treatment of oversedation. Administration of intravenous lipid emulsion was used in one of the cats to facilitate recovery and appeared to be effective in both reducing the depth of midazolam-induced oversedation and significantly reducing the plasma concentration of 1-hydroxymidazolam. The effects after the administration of both treatment modalities on clinical signs and plasma drug concentrations in cats are discussed. The observations suggest that cats may eliminate 1-hydroxymidazolam more slowly than expected; consequently dose adjustments may be required when continuous infusion of midazolam is intended. In addition, intravenous lipid emulsion may facilitate recovery from midazolam oversedation, particularly in cases unresponsive to traditional treatment modalities. However, further investigations are warranted to delineate the efficacy of this modality in the treatment of midazolam oversedation.

Abbreviations: ILE, intravenous lipid emulsion; $MAC_{NM,}$ minimum alveolar concentration corresponding to no movement in response to a noxious stimulus

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Midazolam is a water-soluble benzodiazepine that is used for sedation and as an anesthetic coinduction agent in cats.[21,28,37] Similar to other benzodiazepines, midazolam is considered to cause minimal cardiovascular or respiratory depression and provides good muscle relaxation.[10,35] The rapid elimination half-life of midazolam in dogs (28 to 31 min)[35] and humans (1.5 to 3 h)[33] makes midazolam a more suitable agent for use in continuous infusion than diazepam.[10,33] In humans, midazolam is considered to have a wide margin of safety in comparison to other drugs in this class[33] and is often administered through continuous infusion for patients in the critical care setting.[16,29,34]

Midazolam is a useful adjunctive agent during general anesthesia in dogs,[15,39] rabbits,[17] and goats,[12] primarily through the reduction of the minimum alveolar concentration (MAC) of inhalant anesthetics or as part of a total intravenous anesthetic technique. Continuous infusion of midazolam in cats has been reported in only one prior study;[6] however, plasma concentrations were not measured. At present, there is no published report on its effects on MAC in cats. Therefore, we designed a pharmacokinetic evaluation and a subsequent pharmacodynamic study to investigate the sparing effects of 3 midazolam infusion rates on the minimum alveolar concentration of sevoflurane that prevented movement in response to noxious stimulation (MAC_{NM}) in cats.

Adverse effects from midazolam administration in cats occur infrequently. In some experiments, a wide range of midazolam doses (from 0.05 to 20 mg/kg IV) have been administered to cats.[19,26] Paradoxical behavior such as increased aggression, restlessness, and increased appetite have been described to occur with administration of midazolam even at low doses.[5,19] Sedation, ataxia, and recumbency have also been observed, but treatment is usually neither indicated nor attempted.[6,20,21] During the start of our pharmacodynamics study, the occurrence of prolonged and recurrent sedation in 2 of the 6 cats, despite administration of the specific reversal agent (flumazenil), ultimately prompted discontinuation of the study. The clinical signs and treatment outcomes for those 2 cats are described here.

Case Report

Six healthy, adult research cats (approximately 2 y of age, Class A) underwent a cohort study to determine the

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pharmacokinetics of intravenous midazolam under sevoflurane anesthesia. Cats were current on all routine preventative care measures and deemed healthy according to physical examination and blood analysis. The study was approved by the IACUC of the University of Tennessee, Knoxville, and cats were cared for as described in the *Guide*[23] and USDA regulations[2] in the university's AAALAC-accredited laboratory animal housing facility. Briefly, anesthesia was induced and maintained by using sevoflurane, and a single dose of midazolam (0.3 mg/kg, IV) was administered as a bolus. The cats were maintained under anesthesia, and plasma samples were collected at multiple time points over the next 8 h after administration of midazolam to measure concentrations of midazolam and 1-hydroxymidazolam over time. The midazolam pharmacokinetics profile was analyzed by using pharmacokinetics software (Phoenix WinNonlin 8.0 and Phoenix NLME 8.0, Certara, Princeton, NJ), and the data are being reported in a separate manuscript, which is currently under preparation. Based on the obtained pharmacokinetics data, a 3-compartment model was used to derive a series of infusion rates that would result in steady-state plasma midazolam concentrations corresponding to the peak plasma midazolam concentration after intravenous bolus administration of either 0.2 mg/kg (low dose), 0.4 mg/kg (medium dose), or 0.8 mg/kg (high dose). These doses were selected to evaluate a dose-effect response and were selected in light of the current empirical clinical use of midazolam in cats [5,21,37] and a prior study in dogs.[39]

One month after the pharmacokinetic study, the pharmacodynamic study was initiated to evaluate the sevoflurane MAC_{NM}-sparing effects of the 3 selected midazolam continuous-infusion regimens that were based on the pharmacokinetic modeling. The pharmacodynamic phase was designed as a randomized, blinded, cross-over study using 6 cats. Each cat was assigned to receive all 3 midazolam infusion regimens, with a 7-d washout between treatments. The first cat was assigned to receive midazolam at the high-dose infusion regimen. According to the study protocol, the animal was anesthetized with sevoflurane, intubated, and received mechanical ventilation to maintain end-tidal CO_2 at 35 to 45 mm Hg. The starting end-tidal sevoflurane concentration was set at 3.0% to achieve an adequate plane of anesthesia. Heart rate, ECG, body temperature, end-tidal CO_2, and SpO_2 were monitored by using a multiparameter anesthesia monitor (model S/5, Datex-Ohmeda, Madison, WI). Indirect blood pressure was monitored and recorded every 5 min (Cardell 9402 Veterinary Monitor, Midmark, Tampa, FL). After 60 min of anesthetic equilibration, baseline sevoflurane MAC_{NM} was determined by applying an electrical stimulus to the forearm area, as described in a prior study.[39] A baseline blood sample was obtained before midazolam administration. Intermittent mild hypotension was corrected by using a dopamine infusion to maintain a mean arterial pressure of 60 mm Hg or greater. After determination of baseline sevoflurane MAC_{NM}, based on the pharmacokinetics modeling, a loading dose of midazolam (1.2 mg/kg IV) was administered through a cephalic venous catheter, followed by a series of midazolam infusions (Table 1). According to the same modeling, at 81 min after the start of the initial midazolam infusion, the steady-state plasma concentration was expected to be achieved, and MAC_{NM} redetermination was started for comparison with the baseline values. Venous blood samples were collected through a jugular catheter at 81 min and at MAC_{NM} redetermination time points to associate midazolam plasma concentrations with the percentage decrease in baseline MAC_{NM}. After MAC_{NM} redetermination, midazolam and sevoflurane were discontinued. The total time under sevoflurane anesthesia for this first cat was 5 h, and the total duration of midazolam infusion was 3 h. After discontinuation of the anesthetic agents, the swallowing reflex returned after 10 min, and the cat was extubated with no complications. During the recovery period, although the cat was responsive to touch and sound and although all vital parameters (temperature, heart rate, and respiratory rate) were within normal ranges, the animal seemed heavily sedated even at 60 min after extubation.

To facilitate recovery from oversedation, flumazenil (0.01 mg/kg IV) was administered at 1 h after extubation, after which the cat appeared more alert, sat up sternally, and started to ambulate. Approximately 1.5 h after the first dose of flumazenil, the cat remained in sternal recumbency, but with its head down, and displayed miosis bilaterally, with either active or passive closure of the third eyelids, and no palpebral response. Due to the lack of response to physical stimulation, a second dose of flumazenil (0.01 mg/kg IM) was administered at 2.75 h after the initial dose. At 1 h after the second dose of flumazenil (approximately 5 h after extubation), the cat exhibited moderate ataxia but was able to walk and attempted to eat and drink. In addition, exaggerated grooming behavior, including paw rubbing of the face and eyes, yawning, and licking of the feet was noted at this stage. Approximately 2 h after the second dose of flumazenil (about 8 h after extubation), the cat again appeared sedated, and physical stimulations to arouse it failed. Therefore, the cat was transferred to the Veterinary Teaching Hospital ICU for further evaluation and treatment. In ICU, the cat received a third dose of flumazenil (0.01 mg/kg IV). Throughout the next 18 h in ICU, its condition varied from active to somnolent, but gradually, the periods of alertness and normal physical activity increased in duration. The cat displayed an increased appetite at all feeding times in ICU, without any diarrhea or vomiting. Finally, after 2 d of observation in ICU (approximately 45 h after extubation), the cat's clinical status and behavior were considered normal, and it was discharged with no further concerns. A blood sample was collected at the time of discharge, for analysis of plasma midazolam and 1-hydroxymidazolam concentrations.

The second cat in the pharmacodynamic experiment was assigned to receive the low-dose midazolam infusion regimen. Anesthetic induction, maintenance, monitoring, and sevoflurane MAC_{NM} determinations were performed similarly as described for the first animal. After the baseline sevoflurane MAC_{NM} was determined, a loading dose of midazolam (0.3 mg/kg IV) was administered, followed by the midazolam infusion series (Table 1). At 81 min after the start of the first midazolam infusion, sevoflurane MAC_{NM} redetermination began by using the same methodology as for the first cat, and blood samples were collected at each sevoflurane MAC_{NM} redetermination time point. On completion, sevoflurane and midazolam were discontinued. The total anesthesia time for this cat was 6.5 h, and the total duration of midazolam infusion was 4 h The swallowing reflex returned 3 min after cessation of anesthesia, and the cat was extubated afterward. All vital parameters were within normal ranges; nevertheless, similar to the previous animal, this cat remained overly sedated for several minutes after extubation.

Given the experience with a prolonged recovery in the first cat, flumazenil (0.01 mg/kg IV) was administered more expeditiously in the second cat, at 15 min after extubation. However, no improvement in its clinical status was achieved; therefore, flumazenil was repeated at 20- to 30-min intervals for 2 additional doses. Nevertheless, the cat remained sedated and did not show any improvement even at 2 h after extubation. Next, the cat was transferred to ICU for further observations and

Table 1. Midazolam infusion protocol used in the pharmacodynamic study

Cat	Dose group	Loading dose (mg/kg)	Midazolam infusion series (µg/kg/min)	Targeted midazolam concentration at steady state (ng/mL)	Time to steady-state plasma concentration (min)	Measured 1-hydroxymidazolam at steady state (ng/mL)
1	High	1.2	268 (20 min) 160 (50 min) 112 (11 min)	7816	81	17066
2	Low	0.3	67 (20 min) 40 (50 min) 28 (11 min)	1954	81	6043

Cats received the loading dose intravenously over 30 s, followed by 3 consecutive continuous infusions of midazolam at the indicated rates (and durations). After 81 min, midazolam infusion continued at the third infusion rate for the rest of the experiment to maintain a steady-state plasma concentration.

Table 2. Plasma concentrations of midazolam and 1-hydroxymidazolam during the pharmacodynamics studies for cat 1 (high-dose midazolam infusion) and cat 2 (low-dose infusion)

	Cat 1			Cat 2		
Time of collection	Time (min) from T0	Plasma concentration of midazolam (ng/mL)	Plasma concentration of 1-hydroxymidazolam (ng/mL)	Time (min) from T0	Plasma concentration of midazolam (ng/mL)	Plasma concentration of 1-hydroxymidazolam (ng/mL)
MAC_{NM}	–2	6489	22658	–2	2416	10267
After extubation	—	—	—	136	666	11910
1 h after ILE	—	—	—	270	239	2276
ICU discharge	2700	26	3094	1110	29	3391

ILE, intravenous lipid emulsion; T0, end of midazolam infusion. In both cats, ICU discharge was concurrent with the complete resolution of clinical signs.

supportive care, which included the administration of intravenous lipid emulsion (ILE). Intralipid 20% (Fresenius Kabi, Uppsala, Sweden) was administered at 1.5 mL/kg as a bolus, followed by infusion of 0.25 mL/kg/min for 60 min, according to published guidelines for the treatment of toxicity with local anesthetics.[24,36] At 1 h after ILE administration, the cat appeared more alert and active. Over the next 10 h, its condition varied between active and somnolent. Excitatory behaviors similar to those in the first cat were observed and included obsessive licking, wiping the face and eyes, and yawning. An increased appetite at all feeding times, attention-seeking behavior, and vocalization were noted also. A low-grade cardiac murmur was detected after administration of the Intralipid solution and was suspected to be an indication of fluid overload; however, no abnormal lung sound or respiratory effort was noted. The murmur resolved spontaneously by the following morning. The recovery time of the second cat was significantly shorter than for the first animal, and approximately 19 h after extubation, the animal was released from the ICU with no further concerns.

Materials and Methods

Additional venous blood samples were collected during recovery from anesthesia (after extubation, after administration of ILE, at ICU discharge) to correlate the recovery events with plasma concentrations of midazolam and 1-hydroxymidazolam. All collected blood samples were transferred into lithium heparin tubes (BD Vacuum Phlebotomy Tube, Becton Dickinson, Franklin Lakes, NJ), placed on ice, and then centrifuged (Clay Adams Compact II centrifuge, Becton Dickinson) at 1163 × *g*, 8 min within 60 min of collection. Plasma was separated and stored at –80 °C until analysis. Analysis of midazolam and its metabolite in plasma samples was conducted by using reversed-phase HPLC. The system consisted of a model 2695 separations module and a model 2487 UV detector (Waters, Milford, MA). Separation was achieved on a Waters Symmetry Shield C_{18} column (3.9 × 150 mm; 5 µm) protected by a 5-µm Symmetry Shield guard column. The mobile phase was an isocratic mixture of 0.01 M sodium acetate (pH 4.7) with concentrated glacial acetic acid and acetonitrile (66:34). The solution was prepared fresh daily by using double-distilled, deionized water and was filtered (0.22 µm) and degassed before use. The flow rate was 1.3 mL/min, and UV absorbance was measured at 220 nm. Previously frozen samples were thawed and vortexed. Plasma samples (1 mL) each were placed in 15-mL screw cap tubes and mixed with 250 µL of 7.5 M NaOH and 6 mL of methylene chloride. The tubes were rocked for 30 min and then centrifuged for 20 min at 1302 × g. The organic layer was removed, placed in a clean glass tube, and evaporated with nitrogen gas. The residue was reconstituted in 250 µL of the mobile phase and placed in HPLC vials; and a 100-µL volume injected into the HPLC system. Standard curves for plasma analysis were prepared by spiking nontreated plasma with midazolam and hydroxymidazolam, thus producing a linear concentration range of 10 to 2500 ng/mL. Recovery averaged 94% for both drugs. Intraassay variability ranged from 0.6% to 5.0% for midazolam and 0.8% to 5.7% for hydroxymidazolam; interassay variability ranged from 1.8% to 8.6% for midazolam and 3.9% to 7.6% for hydroxymidazolam. Hydroxymidazolam was not deconjugated for analysis.

Results

Plasma concentrations for midazolam and its primary metabolite, 1-hydroxymidazolam, in the 2 cats in the present study are summarized in Tables 1 and 2. Midazolam and

1-hydroxymidazolam were not detected in plasma prior to the administration of midazolam. At the time of the predicted steady-state (81 min after the start of infusion), plasma concentrations for midazolam were within 10% of the predicted values calculated by using the pharmacokinetic model and remained stable until the end of the infusion. Midazolam infusion resulted in a marked reduction in the MAC_{NM} of sevoflurane by 50% in cat 1 (high-dose midazolam regimen) and 45.4% in cat 2 (low-dose midazolam). During infusion, plasma concentrations of 1-hydroxymidazolam exceeded the plasma concentrations of midazolam and peaked after the end of infusion.

Discussion

The primary limitation in the use of midazolam in cats, particularly in the form of continuous rate infusion, is the lack of published data regarding the pharmacokinetics of midazolam in this species. In humans, several pharmacodynamic models have been described for midazolam and are used to predict sedation scores from a range of doses and their corresponding plasma concentrations.[34,41] According to these studies, plasma concentrations of midazolam between 100 and 500 ng/mL will result in the maintenance of a moderate state of sedation.[16,41] Nevertheless, because of the infrequency or impracticality of measuring plasma concentrations in the clinical setting, inadvertent oversedation of human patients remains an issue of concern.[44] A few published studies in horses,[18] sheep,[40] dogs,[8,38] and alpacas[1] provide some pharmacokinetic–pharmacodynamic information regarding single-bolus administration of midazolam in veterinary species, but even fewer studies address administration through continuous infusion. In one previous study using healthy dogs, a midazolam plasma concentration of 372 ± 235 ng/mL reduced the MAC_{NM} of isoflurane by a maximum of 30%.[39] Dogs that received the highest infusion rate in the study achieved midazolam plasma concentrations of 3583 ± 243 ng/mL, with no further reduction in MAC_{NM}. No adverse clinical effect associated with these plasma concentrations was reported.[39]

In light of the results of the current study, peak plasma concentrations of midazolam in both cats were maintained above the range reported for human sedation in the clinical setting. In addition, midazolam plasma concentration in the cat that received the high-dose infusion was higher than in the aforementioned canine study.[39] Both infusion rates administered to the cats resulted in greater reductions of MAC_{NM} of sevoflurane than that observed for isoflurane in the canine study.[39] In addition, over the course of the midazolam infusion in cats, the plasma concentration of 1-hydroxymidazolam significantly exceeded the concentrations of midazolam, whereas in the canine study,[39] plasma concentrations of 1-hydroxymidazolam remained less than that of midazolam even, at the highest infusion dose. Therefore, after comparing the study in dogs with the current study in cats, it seems that despite stable midazolam plasma concentrations, the cats eliminated 1-hydroxymidazolam at a substantially slower rate than dogs. Although the canine study reported a ceiling effect on MAC reduction at midazolam plasma concentrations of 372 ± 235 ng/mL,[39] the cats demonstrated greater MAC reduction at markedly higher midazolam plasma concentrations. However, the correlation between elevated 1-hydroxymidazolam plasma concentrations and sevoflurane MAC reduction is unknown.

Hepatic enzymes (especially cytochrome P450 3A4) are largely responsible for the phase I metabolism of midazolam, with excretion of its metabolite (1-hydroxymidazolam) in the urine in humans.[33] UDP-glucuronosyltransferase is the enzyme involved in phase II of metabolism, in which 1-hydroxymidazolam undergoes glucuronidation.[30] 1-Hydroxymidazolam is an active metabolite,[3,25,27,45] and although much less potent than midazolam, its accumulation in humans (for example, due to renal disease, chronic high-dose administration of the parent drug, or genetic differences in enzyme activities) has been reported to cause prolonged sedation.[7,30] In another report, elevated plasma concentrations of 1-hydroxymidazolam were detected in critically ill human patients who recovered slowly from midazolam sedation.[29] Plasma samples collected from human patients still sedated 36 h or more after discontinuation of midazolam infusion indicated high plasma 1-hydroxymidazolam concentrations (3121 to 11,525 ng/mL), whereas plasma midazolam concentrations were in the subtherapeutic range (less than 100 ng/mL).[3] Plasma concentrations of 1-hydroxymidazolam in excess of 3100 ng/mL were detected in both cats in the present study. Therefore, in cats, the potential contribution of 1-hydroxymidazolam to prolonged sedation should be considered if midazolam is given as a continuous infusion.

Cats are known for differences in drug metabolism and disposition in comparison to dogs and humans.[9] The most frequently cited difference is a relative deficiency in glucuronidation capability, due to the expression of only 2 isoforms for UDP-glucuronosyltransferase in the liver of cats, compared with 10 isoforms in dogs and 9 in humans.[9,43] In the present study, the sustained plasma concentrations of 1-hydroxymidazolam after discontinuation of infusion may be the result of an overload of the redistribution and elimination capabilities of cats. In contrast, in dogs, 1-hydroxymidazolam is rapidly eliminated through the urine (and bile) after glucuronidation and was detected in plasma samples only when high-dose or continuous infusion of midazolam was used.[8,39] Therefore, our current findings may suggest that although cats are capable of metabolizing midazolam to 1-hydroxymidazolam, the clearance and elimination of the metabolite are likely the rate-limiting step in drug clearance. Although enterohepatic recirculation might be considered as a possible reason for high concentrations of midazolam and 1-hydroxymidazolam, such phenomenon does not occur in humans given midazolam;[11,32] similarly, no data are available that support a potential role for enterohepatic recirculation in maintaining a high concentration of 1-hydroxymidazolam.[32]

Standard treatment for benzodiazepine-induced oversedation or toxicity includes using the reversal agent flumazenil. Flumazenil is a benzodiazepine-specific antagonist that competitively inhibits the binding of midazolam to the GABA receptors in the CNS.[4] Flumazenil is FDA-approved for use as a reversal agent for benzodiazepine sedation in humans. The degree of responsiveness to flumazenil reversal varies among subjects and, for humans, it generally is suggested that flumazenil should be administered in small dose increments to effect.[35] Flumazenil is rapidly metabolized in humans, resulting in a short (1 to 3 h) duration of action,[4,35] and therefore may necessitate administration as a continuous infusion in some patients. Currently, flumazenil is not labeled for veterinary usage; nevertheless, a limited number of pharmacodynamic studies using animal models have provided some data regarding the clinical use of flumazenil in dogs[42] and cats.[13,22] In the present study, the first cat displayed a significant but transient improvement in its activity after flumazenil reversal at an initial dose of 0.01 mg/kg. However, despite a low-dose infusion of midazolam, the administration of several doses of flumazenil in the second cat failed to result in any clinical improvement. The lack of the desired reversal effect after flumazenil administration in the second cat may be due to the earlier administration of flumazenil compared with the first cat

when plasma concentrations of midazolam and 1-hydroxymidazolam were higher.

To our knowledge, this study is the first report of the use of ILE for the treatment of midazolam oversedation. ILE involves a sterile formulation of triglycerides that traditionally is used as parenteral nutrition supplementation for anorexic patients.[36] However, this formulation has been useful in the treatment of acute drug poisoning and overdoses, particularly toxicities associated with intravenous administration of local anesthetics.[31,36] The mechanism of action of ILE is hypothesized to be related to its attraction of lipophilic agents, transiently sequestering the drug in the circulation and speeding delivery to the metabolizing and excreting organs. The efficacy of ILE for toxicities due to agents other than local anesthetics is not established, but some reports suggest a rapid improvement of consciousness in severely obtunded human patients hospitalized due to overdose of various prescription or illicit drugs other than midazolam.[14,31] Current recommendations for drug intoxications indicate the use of ILE for overdose conditions that involve lipid-soluble drugs and that are refractory to standard supportive measures.[24,36] Therefore, in the present study, we selected ILE for treatment of the second cat in light of the persistent oversedation and the lack of response to flumazenil administration.

Subjectively, treatment with ILE appeared to improve the cat's clinical mentation. At 1 h after completion of the ILE infusion, the cat's plasma concentrations of midazolam and 1-hydroxymidazolam had decreased (Table 2). Interestingly, the plasma concentration of 1-hydroxymidazolam decreased most substantially (by 532%) after ILE infusion, suggesting that ILE may have played a greater role in the plasma disposition of 1-hydroxymidazolam than the parent compound. Unfortunately, a comparative sample at a similar time point was not collected from the first cat, and given the differences in midazolam infusion rates, direct comparisons of the recovery time and elimination rates are not possible. Therefore, it is difficult to formulate a conclusion regarding the efficacy of ILE in the treatment of midazolam oversedation.

In summary, midazolam infusion in cats at the rates used in this study resulted in oversedation, and although no other adverse consequences resulted, both cats required extensive hospitalization in ICU for supportive treatment and monitoring. Therefore, further studies are required to determine the optimum dose and infusion rates for midazolam administration in cats, to prevent oversedation and prolonged recovery. Furthermore, our current results suggest a short duration of effect and high variability in responses to flumazenil administration. ILE administration appeared to be effective in facilitating recovery from midazolam oversedation in one cat and was associated with a rapid reduction in the plasma concentration of 1-hydroxymidazolam. Therefore, ILE could be considered for the treatment of oversedation or toxicity with midazolam that is unresponsive to flumazenil. However, further studies are warranted to optimize the dose and duration of treatment with ILE and to determine the effects of ILE on the clearance of midazolam and 1-hydroxymidazolam. In addition, further evaluation of the pharmacokinetics of midazolam and 1-hydroxymidazolam in cats may elucidate the primary mechanism for clearance and the extent to which the potency of the metabolite may contribute to the prolongation of recovery.

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