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RESEARCH PAPER

The effect of midazolam on the end-tidal concentration of isoflurane necessary to prevent movement in dogs

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Abstract

Objective To determine the possible additive effect of midazolam, a GABA_A agonist, on the end-tidal concentration of isoflurane that prevents movement (MAC_{NM}) in response to noxious stimulation.

Study design Randomized cross-over experimental study.

Animals Six healthy, adult intact male, mixed-breed dogs.

Methods After baseline isoflurane MAC_{NM} (MAC_{NM-B}) determination, midazolam was administered as a low (LDS), medium (MDS) or high (HDS) dose series of midazolam. Each series consisted of two dose levels, low and high. The LDS was a loading dose (Ld) of 0.2 mg kg⁻¹ and constant rate infusion (CRI) (2.5 µg kg⁻¹ minute⁻¹) (LDL), followed by an Ld (0.4 mg kg⁻¹) and CRI (5 µg kg⁻¹ minute⁻¹) (LDH). The MDS was an Ld (0.8 mg kg⁻¹) and CRI (10 µg kg⁻¹ minute⁻¹) (MDL) followed by an Ld (1.6 mg kg⁻¹) and CRI (20 µg kg⁻¹ minute⁻¹) (MDH). The HDS was an Ld (3.2 mg kg⁻¹) and CRI (40 µg kg⁻¹ minute⁻¹) (HDL) followed by an Ld (6.4 mg kg⁻¹) and CRI (80 µg kg⁻¹ minute⁻¹) (HDH). MAC_{NM} was re-determined after each dose in each series (MAC_{NM-T}).

Results The median MAC_{NM-B} was 1.42. MAC_{NM-B} did not differ among groups (*p* > 0.05). Percentage

reduction in MAC_{NM} was significantly less in the LDS (11 ± 5%) compared with MDS (30 ± 5%) and HDS (32 ± 5%). There was a weak correlation between the plasma midazolam concentration and percentage MAC_{NM} reduction (*r* = 0.36).

Conclusion and clinical relevance Midazolam doses in the range of 10–80 µg kg⁻¹ minute⁻¹ significantly reduced the isoflurane MAC_{NM}. However, doses greater than 10 µg kg⁻¹ minute⁻¹ did not further decrease MAC_{NM} indicating a ceiling effect.

Keywords anaesthesia, dog, isoflurane, midazolam, minimum alveolar concentration reduction.

Introduction

The potency of inhalational anesthetics is usually evaluated using the concept of the minimum alveolar concentration (MAC), which is the alveolar concentration at which 50% of patients do not respond with purposeful movement to a supramaximal noxious stimulus (Merkel & Eger 1963). Because reflexive, nonpurposeful movement is permissible during MAC determination, the potential for confusion in interpretation of purposeful *versus* nonpurposeful movement exists, and this makes the process somewhat subjective. From a clinical standpoint, determining the lowest alveolar concentration of an anesthetic that abolishes all movement, i.e., MAC-no movement (MAC_{NM}), in response to a noxious stimulus is more relevant. It is

generally accepted that an end-tidal concentration 30–50% greater than the MAC is necessary to abolish all movement in response to noxious stimulation (Eger et al. 1965; Quasha et al. 1980).

Isoflurane causes dose-dependent cardiovascular and respiratory depression; therefore, sedatives and analgesics are often administered concurrently to reduce the dose of isoflurane and, thereby, decrease cardiopulmonary depression (Muir et al. 2003; Solano et al. 2006). Midazolam, a water-soluble benzodiazepine with minimal cardiovascular effects, is occasionally used for sedation and pre-anesthetic medication in dogs (Pieri 1983; Greene et al. 1993). 1'-hydroxymidazolam, the major metabolite of midazolam, is less potent, and other metabolites have insignificant pharmacological effects (Pieri 1983; Stoelting & Hillier 2006a). Although there are no reports on the effects of midazolam on isoflurane MAC or MAC derivatives in dogs, midazolam caused a dose-dependent decrease in halothane MAC in humans (Inagaki et al. 1993) and enflurane MAC in dogs (Hall et al. 1988a). The MAC reducing effects of midazolam may be due to its centrally mediated sedative effects or its spinally mediated antinociception or the combination (Yanez et al. 1990; Sumida et al. 1995; Nishiyama 2006). The antinociceptive activity of isoflurane (Wakai et al. 2005) and midazolam (Schofield et al. 1987; Sumida et al. 1995; Kohno et al. 2006) is mediated via spinal GABA_A receptors, thus, it is expected that midazolam would reduce the amount of isoflurane necessary to prevent motor movements in response to noxious stimulation. In the present study, MAC_{NM} was used to evaluate the interaction between midazolam and isoflurane.

The aim of this study was to determine the effect of midazolam on MAC_{NM} in isoflurane-anesthetized dogs. It was hypothesized that midazolam would decrease the MAC_{NM} of isoflurane in a dose-dependent fashion.

Materials and methods

Animals

Six healthy, adult (2–3 years of age) intact female, mixed-breed dogs (19.1 ± 2.2 kg) provided by a commercial vendor were used in the study. Food was withheld for 12 hours prior to anesthesia, but access to water was allowed. The study was approved by the University of Tennessee Institu-

tional Animal Care and Use Committee (Protocol No. 1633).

Experimental design

Each dog was studied on three occasions using a randomized crossover design, with a minimum of 7 days between experiments. A 6 × 3 table was created with the six rows representing dogs and the three columns representing weeks 1–3. Each treatment was applied to two dogs so that all treatments were represented each week and each dog received all treatments over the 3-week period. After the table was devised, dogs were randomly assigned, one to each row in the table.

Anesthesia

Anesthesia was induced with isoflurane in oxygen delivered via a mask from a circle system. After tracheal intubation, anesthesia was maintained with isoflurane in oxygen (2 L minute⁻¹) using a small animal anesthetic machine (North American Drager, Telford, PA, USA). Dogs were positioned in left lateral recumbency. Ventilation was controlled to maintain the end-tidal carbon dioxide partial pressure (P_{E'}CO₂) between 35 and 45 mmHg (4.7–6 kPa). End-tidal isoflurane (E'ISO) and P_{E'}CO₂ values were monitored with an infrared gas analyzer (Criticare Systems, Waukesha, WI, USA). Samples were drawn from the proximal end of the endotracheal tube at a rate of 150 mL minute⁻¹. The gas analyzer was calibrated at the start of each experiment with the calibration gases supplied by the manufacturer (1% isoflurane in 5% CO₂ and 60% N₂O; Criticare Systems). An 18-gauge (6.3 cm) catheter (Milacath; Mila International, Erlanger, KY, USA) was placed in the right jugular vein to facilitate collection of blood for determination of midazolam and 1'-hydroxymidazolam plasma concentrations, and a second 18-gauge (5 cm) catheter (Milacath; Mila International) was placed in a cephalic vein for infusion of acetated Ringer's solution (3 mL kg⁻¹ hour⁻¹) (Abbott Laboratories, North Chicago, IL, USA) and midazolam. Body temperature was monitored using an esophageal probe (Criticare Systems), and a circulating warm-water blanket and warm air blanket (Bair Hugger; Arizant Inc., MN, USA) were used to maintain body temperature within the normal range (37.5–38.5 °C). Heart rate and ECG were monitored continuously. Arterial blood pressure was monitored continuously

from a 22-gauge (2.5 cm) catheter (Milacath, Mila International) in a dorsal pedal artery, using a new disposable transducer (Baxter Healthcare Corporation, Irvine, CA, USA) and monitor (Criticare Systems). The mid-sternum was taken as the zero point when dogs were in lateral recumbency. All catheters were placed aseptically and no antibiotic was administered. Approximately 45 minutes after induction of anesthesia, and with the $\dot{V}ISO$ held constant at 2.0% for at least 20 minutes, determination of baseline MAC_{NM} (MAC_{NM-B}) was initiated.

MAC_{NM-B} determination

A noxious stimulus (50 V, 50 Hz for 10 ms) was delivered (Grass Instrument Company, Quincy, MA, USA) via two 25-gauge electrode needles inserted subcutaneously 5 cm apart over the mid-ulnar area. Two single stimuli, with a 5-second interval, were delivered initially, followed 5 seconds later by a continuous stimulus of 5 seconds' duration, which was repeated after 5 seconds (Valverde et al. 2003). Withdrawal of a limb or any movement of the head or chewing movements after stimulation was deemed to be a positive response. Twitching of the stimulated limb was not regarded as a positive response. The $\dot{V}ISO$ was then increased or decreased by 0.1 vols% depending on whether the response

was positive or negative, respectively, and the noxious stimulus was re-applied following a 20-minute equilibration period. The lowest $\dot{V}ISO$ that abolished all movements in response to the stimulus was considered the MAC_{NM-B}. The MAC_{NM-B} was determined in duplicate, and the mean value was taken as MAC_{NM-B} for that dog on that treatment day. However, if the difference between the two MAC_{NM-B} values was greater than 10%, a third MAC_{NM-B} was performed and averaged with the first two to attain MAC_{NM-B}.

Drug administration

After MAC_{NM-B} determination, dogs were treated with either a low (LDS), medium (MDS), or high dose series (HDS) of midazolam HCL 0.5% (Hospira, Inc., Lake Forest, Illinois, USA). Each dose series (Table 1) consisted of two loading doses (Ld) and two corresponding constant rate infusions.

Midazolam was administered as follows:

- LDS: An Ld of 0.2 mg kg⁻¹ and a constant rate infusion (CRI) of 2.5 µg kg⁻¹ minute⁻¹ (LDL) followed by an Ld of 0.4 mg kg⁻¹ and a CRI of 5 µg kg⁻¹ minute⁻¹ (LDH).
- MDS: An Ld of 0.8 mg kg⁻¹ and a CRI of 10 µg kg⁻¹ minute⁻¹ (MDL) followed by an Ld of 1.6 mg kg⁻¹ and a CRI of 20 µg kg⁻¹ minute⁻¹ (MDH).

Table 1 Effect of IV midazolam on isoflurane MAC_{NM} in dogs (*n* = 6)

| Midazolam CRI (µg kg ⁻¹ minute ⁻¹) | MAC _{NM-B} * | Time-to-MAC _{NM-B} | MAC _{NM-T} * | Time-to-MAC _{NM-T} | % Change | Plasma midazolam (ng mL ⁻¹) | Plasma H-midazolam (ng mL ⁻¹) |
|---|--------------------------|-----------------------------|--------------------------|-----------------------------|----------------------|---|---|
| Low dose series | | | | | | | |
| LDL 2.5 | 1.40 ± 0.07 ^a | 127 ± 29 ^b | 1.24 ± 0.04 ^c | 146 ± 18 ^e | -11 ± 5 ^f | 129 ± 242 ^h | ND |
| LDH 5 | NA | - | 1.21 ± 0.04 ^c | 136 ± 17 ^e | -12 ± 5 ^f | 211 ± 237 ^h | 114 ± 155 ^k |
| Medium dose series | | | | | | | |
| MDL 10 | 1.47 ± 0.08 ^a | 85 ± 28 ^b | 1.01 ± 0.04 ^d | 134 ± 14 ^e | -30 ± 5 ^g | 372 ± 235 ^h | 94 ± 149 ^k |
| MDH 20 | NA | - | 1.00 ± 0.04 ^d | 117 ± 14 ^e | -30 ± 5 ^g | 788 ± 237 ^{hi} | 158 ± 150 ^k |
| High dose series | | | | | | | |
| HDL 40 | 1.36 ± 0.08 ^a | 163 ± 28 ^b | 1.00 ± 0.04 ^d | 128 ± 14 ^e | -27 ± 5 ^g | 1490 ± 234 ⁱ | 620 ± 147 ^k |
| HDH 80 | NA | - | 0.94 ± 0.04 ^d | 105 ± 16 ^e | -32 ± 5 ^g | 3583 ± 243 ^j | 2424 ± 158 ^l |

CRI, continuous rate infusion; MAC, minimum alveolar concentration; H-midazolam, 1'-hydroxymidazolam; ND, not detectable (less than 7.5 ng mL⁻¹); Time-to-MAC_{NM-B}, time to determine MAC_{NM-B} in minutes from induction to completion of MAC_{NM-B} in duplicate; Time-to-MAC_{NM-T}, time to determine MAC_{NM-T} in minutes from start of CRI to completion of MAC_{NM-T} in duplicate; % Change, calculated from MAC_{NM-B} = [(MAC_{NM-T} - MAC_{NM-B}) / MAC_{NM-B}] × 100; NA, not applicable- MAC_{NM-B} was only determined for the initial dose in each series; LDL, LDH: Low and high doses, respectively, in low dose series; MDL, MDH: Low and high doses, respectively, in medium dose series; HDL, HDH: Low and high doses, respectively, in high dose series.

*The MAC_{NM} data are expressed as raw mean ± SEM. Time-to-MAC_{NM} and plasma concentrations of midazolam and 1'-hydroxymidazolam data are expressed as least square means ± SE of the means. Values with different letters in each column are significantly different (*p* < 0.05).

- HDS: An Ld of 3.2 mg kg⁻¹ and a CRI of 40 µg kg⁻¹ minute⁻¹ (HDL) followed by an Ld of 6.4 mg kg⁻¹ and a CRI of 80 µg kg⁻¹ minute⁻¹ (HDH).

The loading and infusion doses were made up to equal volumes for all the dogs (1.5 mL kg⁻¹ for Ld and 3 mL kg⁻¹ hour⁻¹ for the CRI) using normal saline, administered with a syringe pump (Medfusion 2010i; Medox Inc, Duluth, GA, USA) and delivered through the cephalic vein catheter. Each loading dose was given over 20 minutes, and the CRI was started simultaneously (Hall et al. 1988a).

Determination of the first treatment MAC_{NM} (MAC_{NM-T}) in each dose series was initiated 60 minutes after beginning the CRI and with the E₁SO held constant for at least 20 minutes at the MAC_{NM-B} value. The second Ld and CRI were administered, without a washout period, immediately after determination of the MAC_{NM-T} for the first dose in the series. The MAC_{NM-T} determination for the second dose in the series was initiated 60 minutes after the start of the second infusion.

The MAC_{NM-T} was determined as described for MAC_{NM-B}. A blood sample (3 mL) was collected from the right jugular vein into a lithium heparin tube immediately after the last MAC_{NM-T} determination, and the plasma was harvested and stored at -80 °C prior to determination of plasma midazolam and 1'-hydroxymidazolam concentrations.

Drug analysis

Analysis of midazolam and 1'-hydroxymidazolam in plasma samples was conducted using reversed phase HPLC. The system consisted of a 2695 separations module and a 2487 UV detector (Waters, Milford, MA, USA). Separation was attained on a Waters Symmetry C₁₈ 3.9 × 150 mm (5 µm) protected by a 5 µm Symmetry 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 (69:31). It was prepared fresh daily using double-distilled, deionized water filtered (0.22 µm) and degassed before use. The flow rate was 1.0 mL minute⁻¹, and UV absorbance was measured at 220 nm. The column was at ambient temperature.

Frozen samples were thawed and vortexed. One milliliter of plasma sample was placed in 15 mL screw cap tubes and mixed with 1 mL of H₂O, 250 µL of 7.5 M NaOH, and 5 mL of methylene chloride. The tubes were rocked for 30 minutes and then centrifuged for 20 minutes at 1302 *g*. The

organic layer (bottom layer) was removed and placed into a clean glass tube and evaporated with nitrogen gas. The residue was reconstituted in 150 µL of the mobile phase and placed in HPLC vials; a 100 µL volume was then injected into the HPLC system.

Standard curves for plasma analysis were prepared by spiking dog plasma with midazolam and 1'-hydroxymidazolam, which produced a linear concentration range of 10–2500 ng mL⁻¹. Spiked standards were treated exactly as plasma samples for drug determination. Recovery averaged 94% for both drugs. Intra-assay variability ranged from 0.6% to 5.0% for midazolam and 0.8% to 5.7% for 1'-hydroxymidazolam, while inter-assay variability ranged from 1.8% to 8.6% for midazolam and 3.8% to 7.6% for 1'-hydroxymidazolam.

Statistical analysis

A commercial software program, SAS, version 9.1.3 (SAS Institute, Cary, NC, USA), was used for all analyses. Percentage change in MAC_{NM} was calculated as [(MAC_{NM-T} - MAC_{NM-B})/MAC_{NM-B}] × 100. A mixed model ANOVA was used to determine the effect of treatment on percentage change in MAC_{NM}. Independent variables included in the model were treatment, body weight, time-to-MAC_{NM-T} and week. Dog was included as a random effect in the model. A test for differences in MAC_{NM-B} among treatment groups was performed using the same model, but substituting time-to-MAC_{NM-B} in place of MAC_{NM-T}. The model to determine the effect of treatment on midazolam and 1'-hydroxymidazolam plasma concentration included group, time-to-MAC_{NM-T} and body mass as independent variables. Dog and dog-group interaction were included as random factors in the model. The distributions of residuals from the models were used to evaluate model fit to the data. The assumption that the residuals were normally distributed was evaluated with the W statistic of Shapiro–Wilk. When possible, data were transformed to meet this assumption. Differences in time-to-extubation after cessation of isoflurane and midazolam delivery among treatment groups were evaluated using paired *t* tests. Correlation between plasma concentrations of midazolam and 1'-hydroxymidazolam was determined using the method of Pearson. The MAC data are expressed as raw mean ± standard error of the mean (SEM). Time-to-MAC_{NM} and plasma concentrations of midazolam and 1'-hydroxymidazolam

data are expressed as least square means (LSM) \pm SEM. A two-tailed p -value of <0.05 is considered significant for all tests.

Results

The median MAC_{NM-B} was 1.42 with interquartile ranges of 1.2–1.6%. There was no significant effect of dog, week or body weight on MAC_{NM-B} or MAC_{NM-T}. MAC_{NM-B} was not different among groups ($p > 0.05$). The times to determine MAC_{NM-B} and MAC_{NM-T} did not differ among treatments ($p > 0.05$). MAC_{NM-T} did not differ significantly between the low and high doses in any dose series. MAC_{NM-T} in LDS was significantly different from MDS and HDS; however, MAC_{NM-T} was not different between the latter groups ($p > 0.05$). Percentage decrease in MAC_{NM} ranged from 11 ± 5 in LDL to 32 ± 5 in HDH (Table 1). Percentage change in MAC_{NM} was significantly lower in LDS compared to MDS and HDS; however, it did not differ between MDS and HDS.

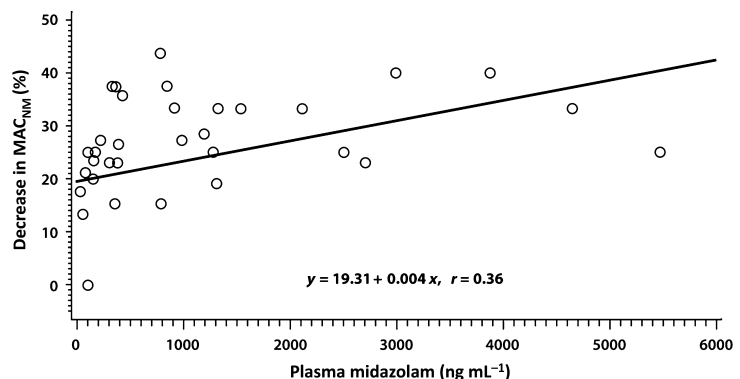
Plasma midazolam concentrations were not significantly different between the LDL and LDH, or between MDL and MDH. However, concentrations were significantly greater in HDH compared to HDL (Table 1). Plasma midazolam concentrations did not differ between the LDS and MDS but were significantly greater in HDS. Plasma concentrations of 1'-hydroxymidazolam were less than the detectable limit of the HPLC technique (7.5 ng mL^{-1}) in four of six dogs in the LDL group; therefore, the results for this group were not analyzed. Plasma 1'-hydroxymidazolam concentrations were significantly greater in the HDH group (Table 1). There was a weak ($r = 0.36$) but significant ($p = 0.03$) correlation between plasma midazolam concentrations and percentage change in MAC_{NM} (Fig. 1). The correlation between plasma 1'-hydroxymidazolam concen-

tration and percentage MAC_{NM} reduction was weak ($r = 0.32$) and not significant ($p = 0.07$). The median value for overall extubation time was 5 minutes (range 3–13 minutes). The time-to-extubation was 7.8 ± 1.4 , 7.1 ± 1.3 and 5.1 ± 1.3 minutes, for LDS, MDS and HDS, respectively, and did not differ ($p > 0.05$) among groups.

Discussion

The results of this study indicate that midazolam, a GABA_A agonist, dose-dependently decreases isoflurane MAC_{NM} in dogs; however, there appears to be a ceiling to this effect as the maximum reduction in MAC_{NM} occurred at the lower dose of the MDS. Percentage decrease in MAC_{NM} in LDS (11 ± 5) was not considered to be clinically significant. The midazolam doses used in the MDS and HDS are greater than doses used clinically (Greene et al. 1993), and were based on doses used in enflurane MAC studies in dogs (Hall et al. 1988a,b). The range of midazolam doses was chosen to determine if dose-response and ceiling effects exist in regard to isoflurane MAC_{NM} reduction, as has been reported for midazolam and enflurane MAC in dogs (Hall et al. 1988a,b). Although midazolam has a relatively short context-sensitive half-time compared with other benzodiazepines, its mean elimination half-life is 77 ± 18 minutes after IV administration to dogs (Court & Greenblatt 1992); thus, it was unrealistic to have a washout period between the infusions in each dose series. In the absence of a washout, the first drug infusion could have influenced plasma concentrations during the second infusion in each dose series, resulting in greater than expected plasma concentrations of midazolam during the second infusion in the series. Therefore, it is more meaningful to interpret the effect of

Figure 1 Diagram depicts the correlation between plasma midazolam concentrations and percentage change in MAC_{NM}. There was a weak but significant ($p = 0.03$) correlation between plasma midazolam concentrations and percentage change in MAC_{NM}.



midazolam on MAC_{NM} based on the plasma drug concentrations.

Traditionally, MAC is used as a measure of the potency of inhalational anesthetics and it is now understood that MAC evaluates the effect of inhalational anesthetics on spinal cord neurons, and is independent of cerebral function (King & Rampil 1994; Yao et al. 2008). Derivatives of MAC, such as the MAC that blocks autonomic responses (MAC_{BAR}), have also been described (Docquier et al. 2003). In the present study, another MAC derivative, MAC_{NM}, defined as the minimum end-tidal concentration of anesthetic preventing motor movement in response to noxious stimulation, was used to evaluate the interaction between isoflurane and midazolam. It is the authors' impression that MAC_{NM} reduces the subjectivity inherent in traditional MAC studies and is more clinically relevant. The median MAC_{NM-B} value (1.42%) in the present study is approximately 16% greater than the traditional isoflurane MAC values reported from the authors' laboratory (1.22 ± 0.22) (Wilson et al. 2008a). In contrast, a comparable endpoint to MAC_{NM} in halothane anesthetized ponies was approximately 60% greater than MAC (Doherty et al. 1997). The greater value for MAC_{NM} relative to MAC in the pony study may be due to a number of factors including differences in species, study methodology, and inhalational anesthetic. The relationship between MAC and its derivatives varies with the inhalational anesthetic. For example, the MAC_{BAR} for desflurane and isoflurane in human patients is approximately 1.3 MAC (Daniel et al. 1998), whereas a MAC_{BAR} of 3.5 MAC has been reported for sevoflurane (Ura et al. 1999). Additionally, the MAC_{AWAKE} is 0.34 MAC for isoflurane and sevoflurane (Eger 2001); yet, a value of 0.59 MAC is reported for halothane (Gaumann et al. 1992).

The maximum isoflurane MAC_{NM} reduction in the present study was approximately 30% at a mean plasma midazolam concentration of 372 ng mL⁻¹. Although MAC_{NM-T} was significantly decreased in the LDS, this decrease was not considered to be clinically significant, as the MAC can vary up to 10–15% among individuals (Stoelting & Hillier 2006b). A number of studies have investigated the MAC-sparing effect of midazolam in humans and dogs, and it appears that the magnitude of MAC reduction is highly variable. In human subjects, a plasma midazolam concentration of 539 ng mL⁻¹ reduced halothane MAC by up to

70% (Inagaki et al. 1993). In dogs, midazolam concentrations of approximately 950 ng mL⁻¹ and 1500 ng mL⁻¹ reduced enflurane MAC by 55% (Hall et al. 1988b) and 60% (Hall et al. 1988a), respectively. The differences in study results could be due to different MAC endpoints, the interaction between midazolam and the different inhalational agents, and variability in midazolam pharmacodynamics among species. It is possible that differences exist in the density and distribution of GABA_A receptors and its subtypes in different species, which may explain interspecies variability in the clinical effects of benzodiazepines. There may also be differences in the mechanisms of spinal cord inhibition among volatile anesthetics. The effect of isoflurane and enflurane on reduction of spontaneous action potential firing in rat spinal cord ventral horn preparations was mediated by GABA_A and glycine receptors; however, the fraction of inhibition mediated by the two receptor systems differed between the two volatile anesthetics (Grasshoff & Antkowiak 2006). Differences in the interaction of inhalational and injectable anesthetics have also been demonstrated; and, in dogs, comparable plasma concentrations of ketamine decreased sevoflurane MAC by 40% (Wilson et al. 2008b) and isoflurane MAC by 27–44% (Solano et al. 2006).

In common with other MAC studies (Hall et al. 1988a), this study demonstrated a ceiling to midazolam's effect on MAC_{NM}, and there was a weak correlation between plasma midazolam and 1'-hydroxymidazolam concentrations and percentage MAC_{NM} reduction. The ceiling effect is thought to be due to saturation of GABA_A receptors after administration of higher doses of midazolam (Hall et al. 1988a; Inagaki et al. 1993). Saturation of GABA_A receptors may occur at lower plasma concentrations of midazolam when isoflurane is being administered concurrently, as both agents bind GABA_A receptors. Thirty-six percent of the reduction in spontaneous action potential firing observed after isoflurane exposure in an *in vitro* ventral horn preparation was mediated by GABA_A receptors (Grasshoff & Antkowiak 2006); thus, the ceiling effect of midazolam on isoflurane MAC_{NM} could be due to a lack of availability of GABA_A receptors in the dorsal horn.

Mean plasma midazolam concentrations of 372 ng mL⁻¹ decreased MAC_{NM} by approximately 30%; however, an almost 10-fold increase in plasma concentrations up to 3583 ng mL⁻¹ (HDS treatment) did not further significantly decrease MAC_{NM}.

In comparison, previous studies in dogs have shown that the ceiling effect on enflurane MAC reduction occurred between 950 and 1500 ng mL⁻¹ (Hall et al. 1988a,b), which is considerably greater than in the present study. This disparity between plasma midazolam concentrations and the onset of a ceiling effect among studies is possibly due to differences in interaction of midazolam with the different inhalational anesthetics (Grasshoff & Antkowiak 2006). Differences in midazolam detection assay could also be considered as another source of variability among different studies.

The decrease in MAC_{NM} by midazolam may be due to its analgesic and/or sedative effects. However, determining the mechanism for the reduction in MAC_{NM} was beyond the scope of this study. Although it is generally accepted that systemically administered midazolam has minimal antinociceptive effects in dogs, midazolam reduced A δ fiber-evoked responses in dorsal horn neurons and C fiber-mediated activity in a model of neuropathic pain in rats (Kontinen & Dickenson 2000). In mice, systemic midazolam demonstrated antinociceptive effects in an inflammatory pain model, but not in a thermal model (Nishiyama 2006). Intrathecal administration of midazolam produced dose-dependent antinociception to thermally evoked pain in rats (Yanez et al. 1990), and intravenous midazolam suppressed noxiously evoked activity of spinal wide dynamic range neurons in cats (Sumida et al. 1995). Therefore, it is plausible that spinally mediated analgesia may play a role in the isoflurane MAC_{NM} sparing effects of midazolam.

In conclusion, midazolam caused a clinically important and statistically significant decrease in the MAC_{NM} of isoflurane in dogs when infused at 10 μ g kg⁻¹ minute⁻¹. Doses greater than 10 μ g kg⁻¹ minute⁻¹ did not cause any further decrease in MAC_{NM}, indicating a ceiling on the MAC_{NM} sparing effect of midazolam.

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