

A pharmacokinetic study of midazolam in dogs: nasal drop vs. atomizer administration

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Abstract

Purpose. The purpose of this investigation was to compare the pharmacokinetics of midazolam following intravenous, intranasal drop, and nasal-atomizer administration in beagle dogs.

Methods: Six animals weighing 9–13 kg were used in a repeated-measure design, group assignment based on route of drug administration. Midazolam (1.5mg/kg) was administered with the delivery route based on group assignment. Blood samples were obtained at baseline and at 1, 3, 5, 7, 10, 15, 20, 30, and 45 min after administration. Cerebrospinal fluid samples (CSF) were obtained at 5 and 10 min after administration. Plasma and CSF concentrations of midazolam were determined by electron-capture gas-liquid chromatography.

Results: Comparison between groups and over time demonstrated that both nasal routes resulted in significantly higher CSF concentrations relative to corresponding plasma levels, and that nasal-atomizer administration produced significantly higher CSF concentrations compared to the drop approach. (*Pediatr Dent* 20:5 321–326, 1998)

Midazolam (Versed,[®] Roche, Nutley, NJ) is a relatively new benzodiazepine that is popular today largely due to its anxiolytic, psychosedative, hypnotic, anticonvulsant, muscle relaxant, and anterograde amnesic properties.^{1–3} Its solubility and unique pH dependent molecular structure account for many of midazolam's desirable characteristics.^{1, 4, 5} In the parenteral preparation, midazolam has a pH of 3.5 and is a water-soluble, relatively nonirritating solution that allows multiple administration approaches, including the nasal approach. At physiologic pH, midazolam is highly lipophilic, which facilitates transport across the blood/brain barrier and accounts for its rapid onset of action. Within the central nervous system (CNS), midazolam has twice the affinity for the benzodiazepine receptor and possesses up to four times the hypnotic potency of diazepam.¹ These effects help explain why midazolam has become the most popular preoperative sedative medication with pediatric anesthesiologists today.^{6, 7} It also accounts for the drug's popularity as a

pharmacologic aid in the outpatient management of pediatric dental patients.⁸

It is well established that premedication reduces adverse psychological and physiologic sequelae in distressed children about to undergo a surgical procedure.⁹ While IV administration is the most effective sedative approach, it is not always the most appropriate for pediatric patients.⁴ Because parenteral administration is a major cause of anxiety, discomfort, and trauma in children, the trend in pediatrics is to avoid injections whenever possible. Although the oral route is more acceptable to children, drug absorption and onset of action are significantly delayed. In addition, the first-pass hepatic metabolism, associated with orally administered midazolam, results in inactivation of up to 70% of the dose prior to reaching the systemic circulation.^{1, 4, 10} Other administration routes, such as sublingual, rectal, and nasal are under consideration as a means to avoid injection sequelae and the first-pass hepatic effect.^{3, 5, 6, 11}

The first study of intranasal midazolam administration in children was conducted in 1988.¹² Subsequent reports have described the transnasal route as an effective alternative to parenteral administration of agents such as sufentanil, ketamine, flurazepam, and triazolam.^{5, 13} Pharmacokinetic study of plasma and cerebrospinal fluid (CSF) concentrations following nasal administration demonstrate the advantage of midazolam's unique molecular properties when using the nasal approach.^{5, 6, 11} Walberg and coworkers found that plasma levels of midazolam were 57% available within 10 min of nose-drop administration.¹⁰ Moreover, these authors suggested that due to direct absorption through the cribiform plate, intranasal administration of midazolam could yield proportionately greater degrees of sedation. It is thought that central communication, through the lymphatics of the nasal mucosa, facilitates midazolam uptake within the CNS beyond that obtained with intravenous or oral administration.^{3–10, 14}

The standard method for nasal administration of midazolam has been in the form of drops via a needleless tuberculin syringe. Use of a metered dose inhaler to deliver medication via a nasal spray is an es-

established technique. Studies with patients who have mild hemophilia compared desmopressin blood levels following nasal spray and drop administration.^{15, 16} Nasal spray was found to deposit the medication more anteriorly in the nasal antrum so that clearance from the nasopharynx occurred more slowly, and resulted in peak plasma levels that were significantly greater than that associated with nasal drop administration.^{15, 17} Because of this information, we hypothesized that nasal-atomizer administration would deposit midazolam in smaller droplets in a manner that would enhance vascular absorption and facilitate uptake within the CNS. Thus, the purpose of our study was to prospectively evaluate the plasma and CSF distribution of midazolam following intravenous, intranasal drop, and nasal-atomizer administration in beagle dogs.

Methods

Protocol

This study was approved by the UTHSCSA Institutional Animal Care and Use Committee. Six healthy beagle dogs (9–13kg) were used on three occasions in a repeated-measure design. Animals were assigned to one of three groups based on the method of midazolam administration, which included intravenous (IV), nasal drop (ND) or the nasal-atomizer (NA) approach. The original protocol called for six animals per group. Power analysis of initial CSF data suggested that significant findings could be obtained by increasing the number of animals per group to 12. Thus, for CSF data, each animal was used twice for an N of 12. Animals were fasted overnight, weighed, and clipped free of hair over both forelimbs prior to venous access. Both cephalic veins were accessed aseptically using an 18-gauge Abbocath® catheter. One vein was used for administration of the anesthetic cocktail and, depending on the group, the midazolam, while the second vein was used for venous blood sampling. Animals were initially anesthetized using 1 cc of an anesthetic cocktail containing: 7.2 mg of xylazine, 2.1 mg of acepromazine, 0.1 mg of atropine sulfate, and 50 mg of ketamine. A baseline blood sample (4 mL) was obtained prior to midazolam administration. Midazolam (1.5 mg/kg) was delivered with the route based on group assignment, and blood samples were collected at 1, 3, 5, 7, 10, 15, 20, 30, and 45 min after dosing. Samples were placed in heparinized tubes. The plasma was separated by centrifugation at 3000 rpm for 10 min and stored at -20°C until assayed by high pressure liquid chromatography (HPLC). Cerebrospinal fluid samples were aseptically obtained

by inserting a 22-gauge, 1 1/2-inch-long, diamond-tip spinal needle into the cisterna magna. The CSF samples (2–3 mL) were obtained at 5 and 10 min after midazolam administration and were stored at -20°C until assayed for midazolam concentration using HPLC. At completion of each experiment animals were given 600 000 units Pen BP prophylactically, followed by a second dose 48 h later. All animals were rested for a minimum of 2 weeks between use in subsequent components of the experiment.

Midazolam administration

For the IV trial, animals received a bolus of commercially available midazolam (5 mg/mL) at a dose of 1.5 mg/kg. For the ND trial, dogs received 1.5 mg/kg of midazolam in a solution containing 15 mg of midazolam per mL. The more concentrated formulation was required to minimize the volume that would have been required with the commercial preparation and thus prevent inadvertent oral administration of midazolam via the nasal route. The solution was prepared by mixing powdered midazolam (provided by Roche Laboratories, Nutley, NJ) with distilled H₂O and adjusting the solution's pH with hydrochloric acid. The drug solution was instilled into the dog's nose while animals were positioned on their side with the head turned and nose pointed up. ND delivery was accomplished by slowly (over 30 s) administering one-half of the total midazolam dose in each nostril using a tuberculin syringe with a narrow 2-cm long polyethylene tube attached. For the NA trial, each dog received 1.5 mg/kg of the midazolam in a solution prepared as above, via a metered-dose inhaler. The solution was sprayed into both nostrils by pumping the inhaler; this delivered 0.13 mL of solution per spray. The volume of midazolam to be administered was calculated based on the animals' weight and the concentration of the solution, with half of the total dose administered in each nostril.

Midazolam assay

Midazolam concentrations in plasma and CSF were determined by HPLC, using an adaptation of a previously reported method.¹⁸ The chromatographic system

TABLE 1. GROUP MEAN (± SD) PLASMA CONCENTRATIONS (C_{MAX} AND T_CMAX) IN DOGS RECEIVING MIDAZOLAM (1.5MG/KG) BY THREE METHODS OF ADMINISTRATION

	<i>C_{max}</i> (ng/mL)	<i>T_Cmax</i> (min)	% Bioavailability
Intravenous	13,335.2 (1205.2)	1.0	—
Nasal Atomizer	971.8 (91.8)*	9.5*	7.3
Nasal Drop	940.7 (108.3)*	10.8*	7.1

* Significant difference from intravenous route, *P* < 0.001.

TABLE 2. GROUP MEAN (\pm SD) CEREBROSPINAL FLUID CONCENTRATIONS (NG/mL), IN DOGS RECEIVING MIDAZOLAM (1.5MG/KG) BY THREE ROUTES OF ADMINISTRATION

	5 min (ng/mL)	10 mins (ng/mL)	% Bioavailability
Intravenous	97.41 (18.4)*	84.4 (9.7)†	—
Nasal Atomizer	8.63 (7.6)	27.13 (12.9)†‡	27.8
Nasal Drop	7.72 (4.1)	15.91 (8.4)†	16.3

* Significant difference from both nasal routes of administration, $P < 0.001$.

† Significant difference within groups from 5- to 10-min time period, $P < 0.003$.

‡ Significant difference from nasal drop administration, $P < 0.02$.

consisted of a model 510 pump, a 710B autosampler, a 15 cm x 4.6 mm Novapak C18 column, a temperature control module, a model 486 UV detector, and a computerized data acquisition system (all Waters Associates, Inc.) The mobile phase was a mixture of acetonitrile/0.02 M monobasic sodium phosphate/triethylamine (250:750:2) adjusted to pH 5.00 with glacial acetic acid, and maintained at a flow rate of 1.4 mL/min. Temperature of the analytical column was maintained at 45°C, and UV detection was at = 245 nm. Sample extraction was accomplished by combining 500 μ L of plasma (or CSF) and 1 mL of 0.2 M sodium borate buffer in a screw-cap glass culture tube and vortex mixing for 30 s. A 5-mL volume of cyclohexane/dichloromethane (7:3, v/v) was then added to the tube and the mixture gently shaken on a reciprocating shaker for 15 min. Following 10 min of centrifugation, the organic phase was transferred to a clean culture tube and evaporated at 35°C under a stream of nitrogen. The sample was then reconstituted with 150 μ L of mobile phase and 20–100 μ L injected on to the analytical column. Retention time for midazolam was 11.8 min. Concentrations were determined by comparing the peak area vs. the concentrations of known standards, ranging from 5 to 1850 ng/mL. Interday coefficient of variation of the assay was 4.8%.

Statistical analysis

The maximum plasma midazolam concentration (C_{max}) and the time to reach the maximum concentration (TC_{max}) were determined and group means compared. Plasma levels were also plotted over time and groups were compared by evaluation of the area under the curve in a manner previously reported.⁴ The plasma bioavailability of midazolam following ND and NA administration was determined by comparing peak levels to the IV route, which was designated 100%. Group data were compared with two-sample Student's t test with significance established at $P < 0.05$. Mean CSF levels were compared over time

and between groups. Group mean data were compared using Student's t test and Wilcoxon's rank-sum test. Significance was established at $P < 0.05$.

Results

Fig 1 represents logarithmic plasma concentrations of midazolam following three methods of administration. Comparison of group mean values revealed significant differences

between IV administration ($P < 0.001$) and both nasal approaches. No significant difference in plasma levels was observed when comparing ND and NA administration.

Table 1 shows the mean maximum plasma midazolam concentrations (C_{max}) and the time required to reach the highest concentration (TC_{max}) for each group. The percent plasma bioavailability of midazolam following ND and NA administration, as compared to peak levels with the IV route (designated 100%), are also shown. Highly significant differences ($P < 0.001$) are evident between the IV route and either nasal method. Differences between the ND and NA technique again were not significant.

Table 2 shows the mean CSF concentrations of midazolam following all three methods of administration. In addition, the percent bioavailability of midazolam within the CSF following ND and NA

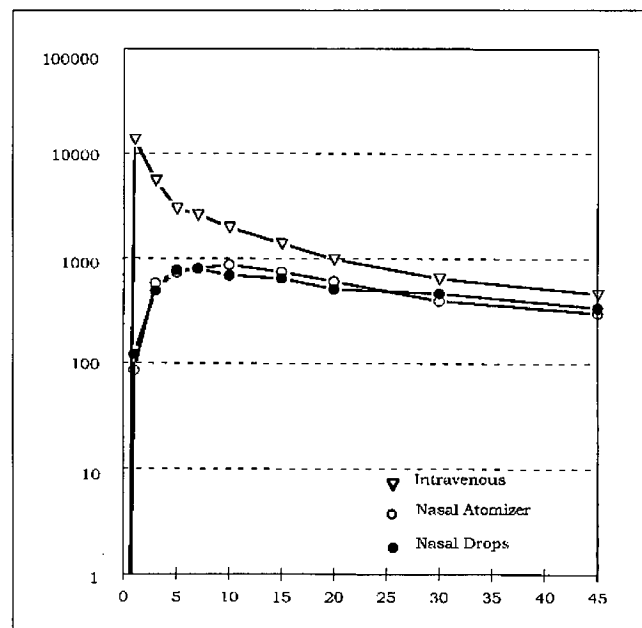


Fig 1. Plasma concentrations versus time following IV, NA, and ND administration of midazolam.

administration is shown as a percent of levels obtained with the IV route. Intragroup comparison revealed a significant change in midazolam concentrations over time within each group ($P < 0.003$). CSF levels decreased with time following IV administration but increased following both nasal routes. At each time interval, the IV route resulted in significantly higher CSF concentrations than either nasal approach ($P < 0.001$). Intergroup comparison of nasal administration demonstrated a significant difference in CSF levels at the 10-min time period where significantly higher concentrations of midazolam were achieved via the NA as compared to the ND approach ($P < 0.02$).

Discussion

Midazolam has a unique pH-dependent biphasic molecular structure that allows multiple administration approaches. The nasal route is a popular alternative which avoids the unpleasantness of parenteral administration and circumvents the first-pass hepatic effect. Midazolam administration via the nasal drop approach has been widely reported.^{5, 6, 11, 19} This investigation is the first to demonstrate that the use of a nasal atomizer enhances CSF bioavailability when midazolam is administered nasally. Our results support those who theorize that intranasal administration may produce proportionately higher concentrations of midazolam within the CNS than other routes.^{3, 6, 9, 10, 14} The possible advantage of atomizer over drop administration, as shown in this study, parallels earlier work demonstrating enhanced drug absorption via the nasal spray technique.¹⁵⁻¹⁷

The olfactory epithelium is a known portal for administration of substances into both the peripheral circulation and the CNS. The specific mechanism by which this occurs was beyond the scope of this investigation. Earlier work, however, may provide some insight concerning the transport of substances between the nasal mucosa, lymphatics, capillaries, neurons, supporting cells, and the subarachnoid space.

The nasal administration of drugs has been practiced since ancient times. Anthropologists have documented the use of hallucinogens and other snuffs or medicines by our early ancestors using this route. We know that the olfactory mucosa lies over a rich network of blood vessels and lymphatics.^{20, 21} Prolonged contact in this area is thought to facilitate absorption of substances by two mechanisms. The first involves absorption through rich vascular and lymphatic networks. Studies have confirmed this mechanism in patients with mild hemophilia and von Willebrand's disease who self-administer desmopressin (DDAVP) nasally. Nasal administration avoids injection and has been shown to produce plasma levels of DDAVP that approximate intravenous administration.^{16, 17} The second mechanism by which nasal absorption is thought to occur is via

pinocytosis, an active-transport process where drug particles are engulfed in a manner not unlike phagocytosis. Pinocytosis by neuronal cells is thought to take place through dendritic protuberances that penetrate the cribiform plate and enter the olfactory bulb.^{21, 22} This may explain why intranasal administration of certain drugs results in unexpectedly high concentrations of drug within CSF.^{20, 22, 23}

Our investigation was based on the premise that substances enter the CSF through the olfactory mucosa by a mechanism unlike that associated with absorption into the systemic circulation. This assumption stems, in part, from animal studies with steroids that demonstrated superior CSF concentrations of drug following nasal administration as compared to the traditional intravenous approach.^{14, 22, 24} Moreover, work with labeled dopamine confirmed the possibility that entry of substances into the blood is not necessarily a prerequisite for its entry to the CNS.²⁰ This investigation, which takes advantage of midazolam's unique pharmacokinetic properties, confirms earlier work suggesting that the nasal administration of certain lipophilic drugs facilitates uptake within the CNS.

This investigation demonstrated that nasal delivery of midazolam produced plasma concentrations approximating 10% of that attained with the IV route. This finding was not in accordance with that of Walbergh and coworkers.¹⁰ They demonstrated that the nasal route achieved plasma concentrations of midazolam that measured 57% of that achieved with IV administration. Payne and coworkers reported bioavailability of midazolam following intramuscular, oral, and rectal administration of 87, 27, and 18% respectively.⁴ The proportionately lower plasma concentrations found in our investigation may relate to a saturation effect of the highly concentrated midazolam required during this animal investigation. Such a saturation effect has been reported before.⁴ A study with pediatric patients found that higher doses of midazolam resulted in mucosal saturation that limited further midazolam absorption.⁴ This concentration-related phenomenon may explain the seemingly low plasma concentrations with the two nasal approaches.

An important finding of this investigation was that the CSF levels observed following either nasal approach did not demonstrate the same saturation phenomenon. These results add to the theory that midazolam need not enter the systemic circulation in order to enter the CNS. This investigation demonstrated that delivery of midazolam via nasal spray resulted in peak plasma concentrations approximating only 7% of the IV route. However, peak CSF concentrations following nasal spray yielded CSF concentrations nearing 30% of that obtained with IV administration. Furthermore, both nasal approaches were shown to produce proportion-

ately greater central uptake of midazolam as compared to absorption systemically. The nasal spray armamentaria seemed to enhance the central uptake of midazolam in the CNS as compared to traditional drop administration.

This investigation is the first to demonstrate possible benefit of using an atomizer for nasal administration of midazolam. The difference in the deposition and clearance following atomizer and drop administration has been previously reported with other drugs.¹⁵ Gamma scintigraphic images demonstrated that spray delivers a drug to a more anterior location where it is less influenced by respiration and thus is less susceptible to oral uptake.¹⁵ Absorption via atomizer is also thought to differ from drop administration in that the atomizer delivers drug to the olfactory mucosa, which is located more superiorly in the nasal cavity at the superior nasal concha, where it is less influenced by inspired air.^{14, 20, 23} For these reasons, one could conclude that a sprayed substance may not migrate from the site of deposition as readily and thus explain enhanced pharmacokinetic uptake within the CNS.

The reader should, however, be cautious in the interpretation and clinical application of these animal data. Although beagle dogs have been utilized previously,^{13, 24} species-specific anatomic differences make direct comparisons with humans impossible and was not the intent of this investigation. Our aim was to determine whether nasal atomizer administration would deposit midazolam in such a way as to enhance vascular absorption and/or facilitate uptake within the CNS. The almost two-fold increase in CSF levels of midazolam following atomizer administration seems beneficial but may potentiate other adverse sequelae. We know that midazolam is capable of producing respiratory depression leading to morbidity and mortality, particularly when administered with narcotics.²⁵ The potential effect on respiratory depression with such an increase in CSF levels is obvious. What is less understood is the direct effect that midazolam may have on neuronal tissues within the CNS. Midazolam administered intrathecally or epidurally has been associated with neurotoxicity in rabbits.²⁶ The enhanced central uptake of midazolam with the atomizer approach could produce CSF levels to the point where neurotoxicity would be more than a theoretical concern. Until such issues are resolved, the prudent practitioner should strictly adhere to published guidelines and monitor the literature regarding the appropriateness of nasal-atomizer delivery of sedative drugs in humans.

Conclusions

1. Nasal drop and atomizer administration of midazolam resulted in significantly higher CSF concentrations of midazolam relative to corresponding plasma levels

2. Nasal atomizer administration produced significantly higher CSF concentrations of midazolam compared to the nasal drop approach
3. CSF concentrations of midazolam increased with time following either nasal approach but decreased with time following IV administration.

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