Pharmacokinetics and pharmacodynamics of intranasal and intravenous naloxone hydrochloride administration in healthy dogs

Brandon M. Wahler DVM
Phillip Lerche BVSc, PhD
Carolina H. Ricco Pereira DVM, MS
Richard M. Bednarski DVM, MS
Butch KuKanich DVM, PhD
Jeffrey Lakritz DVM, PhD
Turi K. Aarnes DVM, MS

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From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210 (Wahler, Lerche, Ricco Pereira, Bednarski, Lakritz, Aarnes); and the Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66502 (KuKanich).

Address correspondence to Dr. Aarnes (aarnes.1@osu.edu).

OBJECTIVE
To evaluate the pharmacokinetics and pharmacodynamics of naloxone hydrochloride in dogs following intranasal (IN) and IV administration.

ANIMALS
6 healthy adult mixed-breed dogs.

PROCEDURES
In a blinded crossover design involving 2 experimental periods separated by a washout period (minimum of 7 days), dogs were randomly assigned to receive naloxone IN (4 mg via a commercially available fixed-dose naloxone atomizer; mean ± SD dose, 0.17 ± 0.02 mg/kg) or IV (0.04 mg/kg) in the first period and then the opposite treatment in the second period. Plasma naloxone concentrations, dog behavior, heart rate, and respiratory rate were evaluated for 24 hours/period.

RESULTS
Naloxone administered IN was well absorbed after a short lag time (mean ± SD, 2.3 ± 1.4 minutes). Mean maximum plasma concentration following IN and IV administration was 9.3 ± 2.5 ng/mL and 18.8 ± 3.9 ng/mL, respectively. Mean time to maximum concentration following IN administration was 22.5 ± 8.2 minutes. Mean terminal half-life after IN and IV administration was 47.4 ± 6.7 minutes and 37.0 ± 6.7 minutes, respectively. Mean bioavailability of naloxone administered IN was 32 ± 13%. There were no notable changes in dog behavior, heart rate, or respiratory rate following naloxone administration by either route.

CONCLUSIONS AND CLINICAL RELEVANCE
Use of a naloxone atomizer for IN naloxone administration in dogs may represent an effective alternative to IV administration in emergency situations involving opioid exposure. Future studies are needed to evaluate the efficacy of IN naloxone administration in dogs with opioid intoxication, including a determination of effective doses. (*Am J Vet Res* 2019;80:696–701)

Evidence from several national health agencies confirms a growing crisis surrounding the abuse of illicit and prescription opioids by humans.[1,2] This rise in opioid abuse is accompanied by the potential risk of accidental exposure of dogs to these drugs in household or occupational settings. Opioid overdose in dogs can lead to severe respiratory and CNS depression.[3,4] From 2009 to 2013, 652 dog exposures to fentanyl were reported to the American Society for the Prevention of Cruelty to Animals; the common clinical signs included sedation, hypersalivation, hypothermia, bradycardia, and ataxia, although no fatalities were noted.[5] Recently reported police dog fatalities attributed to heroin exposure[6] may have, in some instances, resulted from simultaneous exposure to fentanyl or carfentanil that was mixed with the heroin.

Naloxone is a short-acting opioid receptor antagonist with broad opioid receptor affinity and the ability to displace both endogenous and exogenous opioids in a titratable manner.[3,4,7,8] In dogs, naloxone is rapidly absorbed after IV and IM administration and has an apparent wide margin of safety (ie, for doses ranging from 0.005 mg/kg to 10 mg/kg, IV).[8–13] The current recommendation for cardiopulmonary cerebral resuscitation in cats and dogs following cardio-

ABBREVIATIONS

AUC	Area under the concentration-versus-time curve
$AUC_{0-\infty}$	Area under the concentration-versus-time curve from time 0 to infinity
AUC_{0-last}	Area under the concentration-versus-time curve from time 0 to the last measured concentration
AUMC	Area under the first moment curve
$AUMC_{0-\infty}$	Area under the moment curve from time 0 to infinity
C_{last}	Last measured concentration
C_{max}	Maximum observed concentration
IN	Intranasal
λ_z	Terminal rate constant
MRT	Mean residence time
$t_{1/2\lambda}$	Terminal half-life
t_{last}	Time to last measured concentration
t_{max}	Time to maximum concentration

pulmonary arrest associated with opioid administration is to administer naloxone at 0.04 mg/kg, IV, as a reversal agent.[14]

A concentrated formulation of naloxone in an atomizer designed for IN administration is available for use by nonmedically trained individuals to treat people with opioid overdose. The pharmacokinetics of IN naloxone administration have been described in healthy humans[15–18]; this route of administration is associated with acceptable efficacy and bioavailability and minimal adverse effects.[16,18–20] To the authors' knowledge, no studies have been reported on the pharmacokinetics of IN naloxone administration in dogs.

The purpose of the study reported here was to determine the pharmacokinetics of naloxone hydrochloride after IN and IV administration to healthy dogs that had received no other medication and to determine the drug's effects on dog behavior, heart rate, and respiratory rate. We hypothesized that naloxone would be well absorbed following IN administration, with measurable plasma naloxone concentrations in all dogs. We also hypothesized that IN naloxone administration would have minimal effects on the behavior, heart rate, and respiratory rate of healthy dogs.

Materials and Methods

Dogs

Six sexually intact purpose-bred mixed-breed dogs (3 males and 3 females) were included in the study. The median age of the dogs was 6 months (range, 6 to 10 months), and the mean ± SD body weight was 24.8 ± 3.4 kg. A physical examination, CBC, and serum biochemical analyses were performed approximately 1 week prior to the start of the study to ensure that the dogs were in good health. Food was withheld for 12 hours prior to each trial, and dogs were allowed free access to water during that period. All procedures were approved by the Institutional Animal Care and Use Committee of The Ohio State University (protocol No. 2017A00000039).

Experimental design

In a crossover design involving 2 experimental periods separated by a washout period (minimum of 7 days), dogs were randomly assigned by means of a sealed-envelope draw to first receive naloxone hydrochloride IN (4 mg via a commercially available single-dose naloxone atomizer[a]; n = 3) or IV[b] (0.04 mg/kg; 3) and then the opposite treatment in the later experimental period. The doses administered IV and IN were selected on the basis of those recommended for IV administration to dogs during cardiopulmonary cerebral resuscitation[14] and availability for the single-dose naloxone atomizer, respectively.

Two IV catheters were aseptically placed in each dog during each experimental period. A 20-gauge, 3.2-cm catheter[c] was aseptically placed in a cephalic vein for administration of naloxone. A 5F, 20-cm catheter[d] was aseptically placed in a lateral saphenous vein for blood sample collection to determine plasma naloxone concentrations. One observer (BMW), who was not present during drug administration, recorded all observations and collected blood samples for all dogs at baseline (ie, immediately prior to naloxone administration) and after naloxone administration (at 1, 2, 5, 10, 15, 30, 45, 60, and 90 minutes and 2, 4, 8, 12, and 24 hours) for each trial. Heart rate and respiratory rate were obtained by palpation of the femoral arterial pulse for 15 seconds and counting excursions of the lateral aspect of the thorax for 15 seconds, respectively; the presence of any respiratory stertor or stridor was noted. All dogs were also observed for any behavioral changes (ie, postural changes, dysphoria, or excitement) and vomiting. Visual assessments were performed first, followed by measurement of heart rate, then respiratory rate; blood samples were collected last to minimize any effect of blood sample collection on the other variables.

One investigator (TKA) administered naloxone to all dogs immediately following collection of baseline measurements. For the IN treatment, the naloxone atomizer was inserted into 1 naris, then activated by pushing the plunger at the base of the device; following IN administration, each dog's head was stabilized in a neutral position for approximately 30 seconds to minimize any drug loss as a result of dripping from the nares or shaking of the head. Following IV naloxone administration, the catheter in the cephalic vein was flushed with 3 mL of sterile saline (0.9% NaCl) solution.

At baseline and at all subsequent time points, 5- and 6-mL blood samples were collected from the catheter into separate syringes. The 6-mL blood sample was immediately transferred to a sodium heparin blood-collection tube; the 5-mL sample was replaced into the catheter, and the catheter was flushed with 5 mL of saline solution. Blood samples were refrigerated and then centrifuged for 20 minutes at 2,000 × *g* and 4°C within 1 hour after collection. Plasma was harvested and frozen at –80°C until assayed.

Measurement of plasma naloxone concentrations

Plasma naloxone concentrations were determined by means of ultra–high-performance liquid chromatography[e] and tandem mass spectrometry with a triple quadrupole mass spectrometer.[f] The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile at a flow rate of 0.5 mL/min with the following gradient: start with 95% of A, ramp to 5% of A at 0.80 minutes, hold until 1.20 minutes, and ramp to 95% of A at 1.21 minutes with a run time of 2.00 minutes. The column[g] (2.1 mm × 50 mm × 1.8 µm) was maintained at 40°C. The injection volume was 2 µL. The samples were maintained at 5°C in the autosampler. The samples, standards, and quality control samples were prepared identically by the same chemist.

Plasma samples (125 µL) were pipetted into wells of a 48-well plate[h]; 50 µL of internal standard (naloxone-d5 [50 ng/mL] in 200mM ammonium formate with 4% phosphoric acid in water) was added, followed by 175 µL of 200mM ammonium formate with 4% phosphoric acid in water. The plate was then oscillated for 30 minutes at 400 oscillations/min, then centrifuged at 3,500 X *g* for 30 minutes. For plasma extraction, the pretreated plasma samples (350 µL) were each loaded into a sample well of a cation exchange plate[i] (96-well format) and positive pressure (10 to 12 lb/sq in) was applied. Afterward, the sample wells were washed with 100% methanol (300 µL) and eluted with 5% ammonium hydroxide in methanol (50 µL). Deionized water (50 µL) was added to each well. The 96-well plates were then placed in the autosampler. The mass spectrometer was set to positive electrospray, and m/z for qualification and quantification was set as follows (qualifying→quantifying ions): naloxone, 328.08→212.08 and 253.15, respectively; and internal standard (naloxone-d5), 333.18→212.10 and 258.12, respectively. The standard curve was linear from 1 to 500 ng/mL in canine plasma. The accuracy of the assay at 1, 10, 50, and 100 ng/mL (4 replicates at each concentration) was 5%, 4%, –4%, and 4% of the actual concentration, respectively. The coefficient of variation of the assay at 1, 10, 50, and 100 ng/mL (4 replicates at each concentration) was 0.5%, 3%, 3%, and 4%, respectively.

Statistical analysis

The Kolmogorov-Smirnov test was used to determine whether the heart rate and respiratory rate data were normally distributed. Normally distributed data were analyzed with a 2-way ANOVA to compare heart rate and respiratory rate data between the experimental periods (IN vs IV) and at baseline versus subsequent time points after naloxone administration (within each experimental period); a Bonferroni posttest was performed when differences were found. Values of $P < 0.05$ were considered significant.

Pharmacokinetic analysis

Plasma concentration-versus-time and dose data for each dog were subjected to noncompartmental analysis by use of a computer software program[j]. Default values were data weighted as the inverse of the measured plasma naloxone concentration. Noncompartmental analysis was conducted by use of the default program settings,[j] with the exception that all samples (including consecutive samples) with concentrations below the lower limit of quantification were assigned a missing value.

Values for C_{max} and the corresponding t_{max} were obtained directly from the concentration-versus-time curves for each dog after IV administration. The value for λ_z was estimated by use of log-linear regression of time versus the natural logarithm of the plasma naloxone concentration. The slopes of these linear models incorporated at least 3 terminal data points of plasma concentration-versus-time data. The $t_{1/2\lambda}$ was calculated as $(\ln 2)/\lambda_z$. The $AUC_{0\text{-last}}$ was calculated by use of the log-linear trapezoidal rule. The AUC from the time of the last measured concentration to infinity was calculated by C_{last}/λ_z. The $AUC_{0\text{-}\infty}$ was calculated as the addition of the area calculated from C_{last}/λ_z and $AUC_{0\text{-last}}$. The volume of distribution based on the terminal phase after IV administration was determined as the drug dose/(λz X $AUC_{0\text{-}\infty}$), and steady-state volume of distribution after IV administration was calculated as (drug dose/$AUC_{0\text{-}\infty}$) X MRT from time 0 to infinity. Total clearance of naloxone after IV administration was estimated as drug dose/$AUC_{0\text{-}\infty}$. The $AUMC_{0\text{-}\infty}$ was calculated as plasma naloxone concentration X time^2. The MRT after IV administration was estimated as $AUMC_{0\text{-}\infty}/AUC_{0\text{-}\infty}$.

Plasma concentration-versus-time data for IN naloxone administration were analyzed in a similar manner as the data for IV administration to obtain the pharmacokinetic values, except that all values used in the analysis were those pertaining to IN administration. Bioavailability of naloxone after IN administration was calculated as the AUC after IN administration/AUC after IV administration, after correction for the different doses. Pharmacokinetic parameters were tabulated for each dog by route of administration and reported as mean ± SD.

Results

Pharmacokinetic findings

The mean ± SD delivered naloxone dose for IN administration via the fixed-dose naloxone atomizer was 0.17 ± 0.02 mg/kg (range, 0.14 to 0.19 mg/kg) and for IV administration was 0.040 ± 0.001 mg/kg. Plasma concentration-versus-time curves following IN and IV administration of naloxone were determined up to 250 minutes, at which time plasma concentrations for both routes were below the lower limit of detection **(Figure 1)**. The results of the noncompartmental analysis of plasma naloxone concentrations over time following IN and IV administration were summarized **(Table 1)**.

Pharmacodynamic findings

Heart rate and respiratory rate data were normally distributed; these rates did not change between time points following administration of naloxone and did not differ between administration routes. No behavioral changes or vomiting were noted at any time during the study, and no evidence of nasal congestion (ie, stertor, stridor, or other abnormal respiratory sounds) was noted.

Discussion

The pharmacokinetics of IN naloxone hydrochloride administration had not been previously established for dogs. The present study revealed that, following IN administration, naloxone was rapidly absorbed, with a short lag time of 2.3 minutes to detection of naloxone in plasma samples. Studies[17,21] in

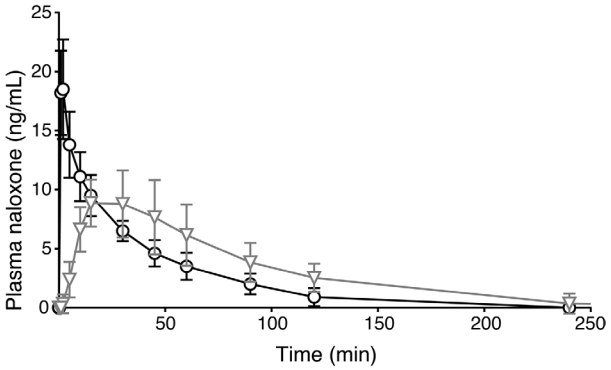

Figure 1—Mean plasma naloxone concentrations immediately before (baseline; 0 minutes) and at various points following IN (4 mg via a fixed-dose naloxone atomizer; individual dose range, 0.14 to 0.19 mg/kg; triangles) and IV (0.04 mg/kg; circles) administration of naloxone hydrochloride to 6 healthy dogs in a randomized crossover design involving 2 experimental periods (n = 3 dogs/administration route/period) separated by a 7-day washout period. Error bars represent SD.

humans show a C_{max} of 5.3 to 6.02 ng/mL and a t_{max} of 15 to 30 minutes following IN administration of 4 mg of naloxone, which are comparable to the results of the present study. The difference in $t_{1/2\lambda}$ between humans[17,21] (1.7 to 2.2 hours) and the dogs in our study (approx 0.8 hours) may be attributable to interspecies differences in the metabolism and clearance of naloxone.

Intranasally administered naloxone had a similar $t_{1/2\lambda}$, compared with that following IV administration. The naloxone dose administered IN to each dog was approximately 4 times the dose administered IV. However, the C_{max} was approximately 50% lower following IN administration than the C_{max} following IV administration. Although the investigators did not observe any swallowing or dripping from the nose of dogs following IN administration, it is possible that loss via these routes could have resulted in reduced absorption. Genetic studies[22,23] of humans and laboratory animals have identified the presence of cytochrome P450 enzymes in the nasal mucosa, which could also potentially decrease absorption of naloxone following IN administration.

Naloxone absorption and distribution in the present study were likely affected by the administration route (IN vs IV); because of the vascularity of the nasal mucosa and its proximity to the cranial nerves, IN administration may have resulted in CSF naloxone concentrations that were higher than measured plasma naloxone concentrations.[24–26] Atomization of naloxone via the specialized delivery device[a] used in the present study creates small droplets that when administered IN could lead to better absorption by the CNS, through dispersion and coverage of the nasal mucosa and local transport via the olfactory and trigeminal nerves, than achieved through other routes of administration.[24,25] We did not investigate IN administration of the injectable formulation of naloxone; the absorption and resulting plasma concentrations following IN administration of that formulation may differ from those obtained when administering the atomizer formulation.

Table 1—Mean ± SD pharmacokinetic values from noncompartmental analysis of plasma naloxone concentrations over time following IN (4 mg via a fixed-dose naloxone atomizer; individual dose range, 0.14 to 0.19 mg/kg) and IV (0.04 mg/kg) administration of naloxone hydrochloride to 6 healthy dogs.

Parameter	IN	IV
R^2	0.997 ± 0.002	0.991 ± 0.007
λ_z (1/min)	0.015 ± 0.002	0.019 ± 0.004
$t_{1/2\lambda}$ (min)	47.4 ± 6.7	37.0 ± 6.7
t_{lag} (min)	2.3 ± 1.4	—
t_{max} (min)	22.5 ± 8.2	—
C_{max} (ng/mL)	9.3 ± 2.5	18.8 ± 3.9
t_{last} (min)	140 ± 49	110 ± 15
C_{last} (ng/mL)	2.5 ± 1.2	1.4 ± 0.3
$AUC_{0-\infty}$ (min•ng/mL)	841 ± 326	657 ± 147
AUC%	—	11.4 ± 1.8
$AUMC_{0-\infty}$ (min^2•ng/mL)	67,856 ± 31,300	32,217 ± 12,755
MRT (min)	79.3 ± 9.2	49.0 ± 9.0
F (%)	32 ± 13	—
V_{ss} (L/kg)	—	3.0 ± 0.6
Cl (mL/min/kg)	—	65.0 ± 13.7
Vd_z (L/kg)	—	3.4 ± 0.5

— = Not applicable. AUC% = Percentage of the AUC that was extrapolated. Cl = Clearance. F = Bioavailability. R^2 = Coefficient of determination. t_{lag} = Time delay between naloxone administration and first observed concentration. Vd_z = Volume of distribution during the terminal phase after IV administration. V_{ss} = Volume of distribution at a steady state.

Although the half-life of a given drug can differ slightly between individuals and species,[20,27] it should be similar within a species. However, a previous study[9] in dogs that involved a radioimmunoassay to measure plasma naloxone concentrations following IV administration of the drug (5 mg/kg) showed a longer mean half-life than obtained following IV administration in the present study. That study[9] was conducted in halothane-anesthetized dogs, which may have altered the pharmacokinetics (ie, clearance) of naloxone because of decreased cardiac output and altered blood flow to the liver and kidneys relative to that in conscious dogs.

There were no notable changes in the heart rate, respiratory rate, or observed behavior of dogs following IV (0.04 mg/kg) or IN (4 mg) administration of naloxone in the present study. This was not surprising because naloxone is an opioid receptor antagonist with no intrinsic efficacy following receptor binding. Previous studies[28,29] of humans who had received no other medications showed minimal pharmacodynamic impact, with no notable changes in heart rate and minimal changes in blood pressure, following naloxone administration. The pharmacodynamics may differ when naloxone is administered to a dog that has previously been administered or accidentally exposed to opioid receptor agonists (eg, reversal of acute opioid effects such as bradycardia, respiratory depression, and hypotension).[12,30,31]

Behavioral changes in humans following IN naloxone administration include restlessness, irritability, and excitement.[32] Intranasal administration of naloxone can also lead to nasal congestion in humans.[33] No behavioral changes or nasal congestion were noted in the dogs of the present study following naloxone administration via either route. In addition, IN administration appeared to be tolerated by the dogs, with minimal need for restraint during and after administration.

Additional studies are needed to evaluate the efficacy of IN naloxone administration for reversing the effects of opioid intoxication in dogs, including a determination of effective doses. In humans,[34] doses as low as 0.005 mg/kg, IV, have been used clinically to reverse morphine-induced respiratory depression following surgery. In dogs recovering from anesthesia, naloxone doses as low as 0.01 mg/kg, IV, are effective in reversing opioid-associated CNS depression.[35]

A limitation of the present study was that a small (n = 6) homogeneous group of purpose-bred mixed-breed dogs was used, which may have limited the generalizability of our findings to other populations of dogs. Genetics have been shown to play a role in drug metabolism in dogs.[36,37] In addition, anatomic variation among individual dogs (eg, nasal conformation) could lead to differences in absorption and metabolism, especially with respect to pharmacokinetics of drugs administered IN.

Naloxone was rapidly absorbed in the dogs of the present study, with clinically useful bioavailability following IN administration by use of a commercially available naloxone atomizer. Plasma $t_{1/2\lambda}$ was similar following IV and IN administration. Given the absence of noted adverse events and the need for minimal restraint associated with IN administration in the dogs in the present study, the naloxone atomizer may represent an effective alternative to IV administration in emergency situations involving opioid exposure.

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The authors declare that there were no conflicts of interest.

Footnotes

a. Narcan nasal spray (4 mg), Adapt Pharma Inc, Radnor, Pa.
b. Naloxone hydrochloride injection (0.4 mg/mL), Hospira Inc, Lake Forest, Ill.
c. Abbott Laboratories, Abbott Park, Ill.
d. MILA International Inc, Florence, Ky.
e. Acquity Prominence UPLC, Waters Corp, Milford, Mass.
f. Xevo TQ-S, Waters Corp, Milford, Mass.
g. Acquity UPLC HSS T3, 1.8 µm, Waters Corp, Milford, Mass.
h. CytoOne 48-well TC plate, USA Scientific, Ocala, Fla.
i. Oasis PRIME MCX 96-well µElution plate, Waters Corp, Milford, Mass.
j. Phoenix WinNonLin, version 8.1, Cerata, Princeton, NJ.

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