Assessment of a von Frey device for evaluation of the antinociceptive effects of morphine and its application in pharmacodynamic modeling of morphine in dogs

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Objective—To assess the use of a von Frey device as a mechanical nociceptive stimulus for evaluation of the antinociceptive effects of morphine in dogs and its potential application in the pharmacodynamic modeling of morphine in that species.

Animals—6 healthy Beagles.

Procedure—von Frey thresholds were measured in all dogs before and at intervals after they received no treatment (control dogs) and IV administration of morphine sulfate (1 mg/kg; treated dogs) in a crossover study. The von Frey device consisted of a rigid tip (0.5 mm in diameter) and an electronic load cell; the operator was unaware of recorded measurements.

Results—Application of the von Frey device was simple and well tolerated by all dogs and caused no apparent tissue damage. No significant changes in thresholds were detected in the control dogs at 8 hourly measurements, indicating a lack of acquired tolerance, learned aversion, or local hyperalgesia. When assessed as a group, treated dogs had significantly high thresholds for 4 hours following morphine administration, compared with baseline values; individually, thresholds decreased to baseline values within (mean ± SE) 2.8 ± 0.6 hours. The maximal effect (change from baseline values) was 213 ± 43%, and the plasma morphine concentration to achieve 50% maximal effect was 13.92 ± 2.39 ng/mL.

Conclusions and Clinical Relevance—Data suggest that, in dogs, evaluation of the antinociceptive effect and pharmacodynamic modeling of a dose of morphine sulfate (1 mg/kg, IV) can be successfully achieved by use of a von Frey device. (*Am J Vet Res* 2005;66:1616–1622)

Morphine is the prototypical opiate analgesic, which interacts with μ and κ opiate receptors to exert its analgesic effect. Despite numerous studies[1-13] of morphine in dogs, the efficacy of morphine has not been objectively evaluated. Several studies[1,3-12] have been undertaken to evaluate pharmacokinetic parameters following IV administration of a bolus of morphine to dogs; the results appear to be relatively consistent in that morphine has a short elimination half-life (0.9 to 1.6 hours), rapid clearance (41 to 85 mL/min/kg), and a large volume of distribution (3.6 to 7.2 L/kg). **Morphine-6-glucuronide (M6G)** is a metabolite of morphine that has various degrees of analgesic activity in humans.[14-16] Results of previous studies[8,13] have indicated that very low amounts of M6G are produced from the metabolism of morphine in dogs; therefore, M6G is unlikely to contribute to analgesic effects associated with morphine administration.

To the authors' knowledge, a pharmacodynamic model to describe the relationship of plasma morphine or M6G concentrations with antinociceptive effects in dogs has not been proposed. The lack of dose titration data or pharmacokinetic-pharmacodynamic evaluations of opiates in dogs is probably attributable to a lack of reliable, consistent, and humane outcome measures for assessment of antinociception or analgesia. von Frey devices have been used to assess antinociceptive effects of drugs in laboratory settings, generally in rodents, and also to assess the analgesic effects of drugs in human patients.[17-19] The only reports[20-22] of the use of von Frey-like devices in dogs have described purpose-built devices, which were successfully used in studies of preemptive analgesia. A device producing a mechanical stimulus, such as the von Frey device, stimulates both Aδ- and c-fiber nociceptors, which are also responsible for encoding naturally occurring pain.[23] Clinically relevant opiate dosages primarily act on c fibers and only act on Aδ-fiber input to the spinal cord when administered in high doses.[23] Therefore, increases in von Frey thresholds following morphine administration would be expected to primarily affect c-fiber input. A von Frey device consists of a tip that is applied to the skin surface with gradually increasing force to create a noxious stimulus. In some von Frey devices, fine hairs are used that bend at a maximum force and larger hairs are used to retest until the subject reacts prior to the hair bending. In electronic devices, a rigid tip is used and the force that is applied is measured by use of an integrated load cell. In humans, the end point of the reaction is determined from a verbal communi-

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cation that the stimulus is perceived as noxious. The end point is reached in animals when the individual reacts or withdraws.

The purpose of the study reported here was to assess the use of a von Frey device as a mechanical nociceptive stimulus for evaluation of the antinociceptive effects of morphine in dogs and its potential application in the pharmacokinetic-pharmacodynamic modeling of morphine in that species. Our hypotheses were that repeated use of the von Frey device would not produce changes in thresholds over time in untreated control dogs, von Frey thresholds would increase in dogs following IV administration of a bolus of morphine, and von Frey thresholds could be used to develop a pharmacodynamic model to relate plasma morphine concentrations to changes in nociceptive thresholds in dogs.

Materials and Methods

Animals—Six healthy Beagles (3 males and 3 females; 4 to 5 years old) were used for this study. These dogs had not been included in any previous studies involving a nociceptive stimulus. Dogs were housed in runs prior to the study and fed a commercial dog food.[a] On any given day, 2 dogs were assessed; the study had a crossover design with an interval of at least 1 week between each phase. Experimental measurements were performed in an isolated, noise-free, temperature-controlled room that was illuminated with artificial lighting to minimize distractions and day-to-day variability in data collection. The North Carolina State University Institutional Animal Care and Use Committee approved the study.

von Frey device—The von Frey device[b] was built to requested specifications and consisted of a load cell, handle, recording device, and tip (**Figure 1**). The tip was custom built from a 0.5-mm-diameter polypropylene tip,[b] which was completely filled with an epoxy putty.[c] The 0.5-mm tip was then placed inside the proximal part of a 1,000-µL pipette tip[d] and filled with epoxy putty to increase the rigidity. The diameter of the tip and surface area of pressure applied was consistent throughout each study phase. The load cell was calibrated from 100 to 1,000 g. The recording device recorded the maximum weight applied to the tip. The operator controlled the rate of application and was trained in constant rate of application.

von Frey threshold testing—von Frey threshold measurements were made by the same operator (BK), who was unaware of the actual readings obtained (another operator recorded the thresholds). All measurements were made in dogs that were standing with minimal restraint. Pressure was exerted until a withdrawal or escape movement was made, vocalization occurred, or the maximum weight (1,000 g) was applied. If maximum weight was applied, the operator was notified to stop to reduce the risk of tissue injury and the weight recorded as 1,000 g. A simple withdrawal reflex on first touch of the device tip to the footpad was not accepted as an end point.

Initially, various parts of the body were assessed for ability to produce consistent results. Areas assessed included the tibial tuberosity, spinous processes, tail base, pinna, carpal pad, metatarsal bones, olecranon, and spine of the scapula. Additionally, a 3-mm-diameter tip was assessed, but weights applied to untreated control dogs exceeded 1,000 g. Use of the carpal pad provided the most consistent results, and all subsequent experiments were performed on the area of the carpal pads by use of a device with a 0.5-mm tip (**Figure 2**).

Visual examination for tissue damage caused by pressure exerted via the von Frey device was performed during each phase of the study. The carpal pads were examined for redness, swelling, signs of pain on palpation, bleeding, and exudate during and for 24 hours after each evaluation was complete. Additionally, the gait of each dog was assessed for signs of lameness at the conclusion of the evaluation period and 24 hours after the completion of each crossover.

At each time point, 3 measurements were obtained from the left and right forelimbs. The von Frey thresholds were expressed as a percentage change from baseline values. Baseline values for the control dogs were established as the time 0 measurements; all subsequent values were expressed as a percentage change from time 0 measurements. Baseline values for the treated dogs were obtained prior to drug administration (time 0); all subsequent values were expressed as a percentage change from baseline measurements. All percentage-change values were calculated for each individual dog and then pooled for group values.

Study design—The study had a 2-way crossover design in which all dogs were evaluated after receiving no treatment (control dogs) and after treatment with morphine (treated

Figure 1—Photograph of a von Frey device with a load cell built into the handle and the tip used to evaluate the antinociceptive effects of morphine in dogs.

dogs). The interval between phases of the study was at least 1 week, and the order consisted of the control group followed by the treatment group. The control dogs received no medication (or sham treatments); the von Frey device was applied to the carpal pad of the left and right forelimbs, and von Frey threshold measurements (3/limb) were obtained hourly for 8 measurements. Treated dogs received a bolus of morphine sulfate[e] (1 mg/kg, IV); in the manner described for the control dogs, von Frey threshold measurements were obtained beginning at time 0 (ie, prior to drug administration) and hourly for 4 hours (experiments were terminated at this time because threshold values had returned to baseline threshold values) for a total of 5 measurements. All dogs were subjectively assessed for signs of sedation.

Morphine sulfate was administered IV as a bolus into the cephalic vein (duration of administration, 15 seconds); each dog received 1 mg of drug/kg (equivalent to 0.75 mg of morphine base/kg) in this phase of the study. Blood samples (7 mL) were collected via jugular venipuncture prior to morphine administration and hourly (after completion of each of the von Frey threshold evaluations). Blood samples were collected into evacuated glass tubes containing lithium heparin.[f] The tubes were placed on ice and centrifuged at 1,000 × *g* for 10 minutes; plasma was separated and stored frozen at –70°C. All plasma samples were analyzed within 30 days of collection.

Plasma drug concentrations—Plasma samples were analyzed for morphine and M6G via a **high-pressure liquid chromatography (HPLC)** method with electrochemical coulometric detection. The HPLC system consisted of a pump,[g] degasser,[g] autosampler,[g] and electrochemical coulometric detector.[h] A 4.6 × 150-mm, 5-µm phenyl column[i] maintained at 40°C was used to achieve separation. The mobile phase consisted of 95% 0.01M acetate buffer[j] with 0.1% triethylamine[j] and 5% acetonitrile[j] (pH adjusted to 4.5 with glacial acetic acid[j]). A gradient was programmed in the following manner: 0 to 8 minutes, 100% mobile phase; 8 to 11 minutes, ramped to 85% mobile phase and 15% acetonitrile; 11 to 14 minutes, ramped to 100% mobile phase; and 14 to 20 minutes, 100% mobile phase. Retention times for M6G and morphine were 5.5 and 7.5 minutes, respectively. The detector settings were as follows: guard cell, +750 mV; cell 1, +300 mV; and cell 2, +450 mV (cell 2 quantified by use of computer software[k]).

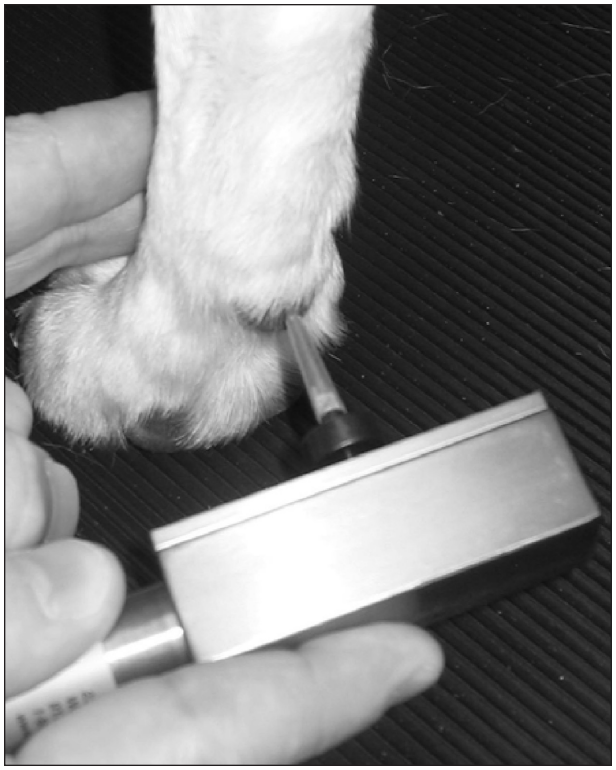

Figure 2—Photograph of the application of the von Frey device to the left carpal pad of a dog.

Each plasma sample (1 mL) was treated with 1 mL of 0.2M borate buffer[j] (pH, 9.0), vortexed, and then treated with 0.4 mL of 0.1M 1-pentanesulfonic acid[j] and vortexed again. Solid phase extraction cartridges[l] were conditioned with 1 mL of HPLC-grade water and 1 mL of 100% methanol[j]; the plasma mixture (2.4 mL) was loaded onto the extraction cartridges, washed with 1 mL of HPLC-grade water, and eluted with 1 mL of 100% methanol. The eluate was evaporated under a nitrogen stream at 40°C and reconstituted with 0.2 mL of mobile phase. The injection volume was 0.05 mL.

Calibration curves were made daily by fortifying a pooled sample of canine plasma (obtained from the study dogs) with known amounts of morphine hydrochloride[m] and M6G,[m] which were processed in the same manner as the experimental plasma samples. Calibration curves were accepted if the coefficient of determination (r^2) was > 0.99 and the measured values were within 15% of the actual values (except the highest and lowest points, which had to be within 20%).[24] Control samples consisting of fortified canine plasma were assessed at the beginning of the day, intermittently during the day, and at the end of the day; that day's run of experimental samples was accepted if values for the control samples were within 15% of the actual value.

Statistical analyses and pharmacokinetic and pharmacodynamic modeling—Statistical analyses were conducted by use of a computer software program[n]; significance was set at a value of $P < 0.05$. The Mann-Whitney rank sum test was used to evaluate differences in baseline thresholds between the control dogs and the treated dogs. The Kruskal-Wallis analysis of variance was used to evaluate differences across time points within each group because the variances were not equal. If differences were found, the Dunn test for multiple comparisons to time 0 (for the respective group) was used to discriminate the time points that were significantly different. Pharmacokinetic and pharmacodynamic modeling were conducted in a standard 2-stage design by use of a computer program.[o] Pharmacodynamic modeling calculated a concentration-effect relationship expressed by the following equation:

$$E = (E_{MAX} \bullet C) / (C + EC_{50}),$$

where E is the effect (% change in von Frey threshold from baseline value), E_{MAX} is the maximum effect, C is the plasma concentration of morphine (ng/mL), and EC_{50} is the concentration of morphine associated with a 50% maximal response.

Results

The use of the von Frey pressure device was well tolerated by all dogs, and there was no evidence of a learned behavior or aversion to its use. von Frey thresholds measured at the tibial tuberosity, spinous processes, tail base, pinna, metatarsal bones, olecranon, and spine of the scapula were either variable or exceeded the maximum pressures in dogs receiving no treatment. For example, the mean ± SE baseline von Frey threshold in the region of the metatarsal bones in untreated dogs was 442.1 ± 34.3 g. The most consistent results were obtained at the carpal pad. The baseline von Frey threshold at the carpal pad for the con-

trol and treated dogs was 185.0 ± 4.1 g and 209.0 ± 12.9 g, respectively. These values were not significantly different. There was no significant difference between threshold values obtained from the left and right carpal pads; therefore, the values were pooled for analysis. von Frey thresholds did not vary significantly over time in the control dogs, either individually or as a group, and none of the dogs reached maximum thresholds (**Figure 3**).

The most common response to application of the von Frey device to the carpal pad was active withdrawal of the limb; 1 dog would occasionally vocalize. The maximum weight applied (1,000 g) was attained at the 1-hour time point in 3 of the 6 dogs after treatment with morphine. The maximum weight was also attained in 1 of those 3 dogs at 2 hours after treatment with morphine. Maximum weight was not attained in any of the dogs at 3 or 4 hours after morphine treatment. There was no evidence of tissue injury or lameness at any time prior to, during, or after the study for any of the dogs in either group. Transient dimples were detected in the footpads after the measurements but disappeared prior to the next hourly measurement. There was no evidence of hyperesthesia or tolerance to the stimulus in the control dogs.

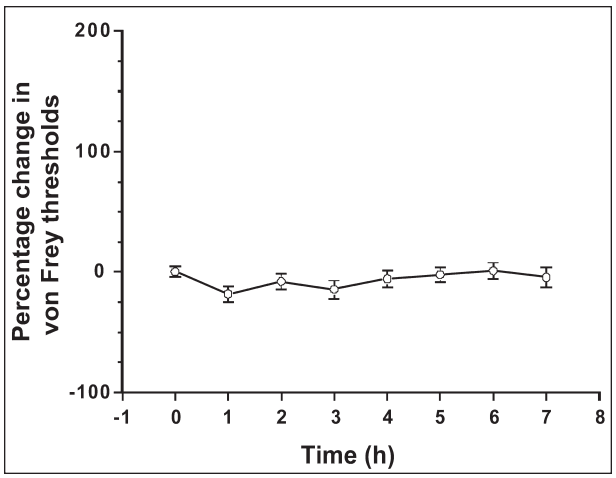

Figure 3—Mean ± SE percentage change in von Frey thresholds (measured at the carpal pad) from baseline values (time 0) assessed hourly in 6 dogs that received no treatment (control phase of a crossover study). Mean threshold values did not differ significantly from baseline at any time point.

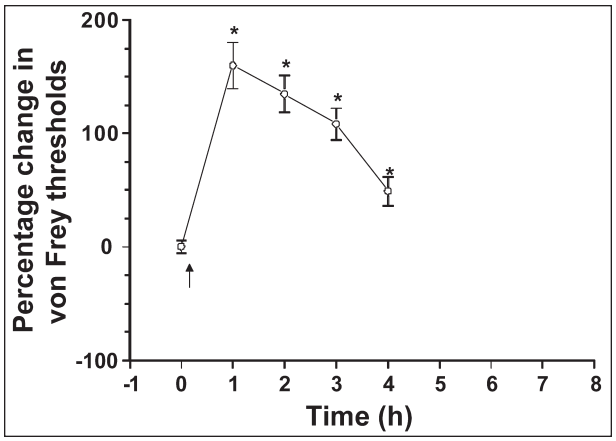

Figure 4—Mean ± SE percentage change in von Frey thresholds (measured at the carpal pad) from baseline values (obtained prior to drug administration; time 0) in 6 dogs that received IV administration (arrow) of a bolus of morphine sulfate (1 mg/kg; treatment phase of a crossover study) during a 4-hour evaluation period. No data were collected after 4 hours. *Mean threshold value significantly ($P < 0.05$) different from baseline at this time point.

Following IV administration of morphine, all of the dogs vocalized briefly (approx 30 seconds after the injection was completed). None of the dogs vomited, but all were sedated for 7 to 12 hours after administration of morphine. Postures, as determined visually, varied from apparently resting comfortably to sitting hunched in the back corner of the kennel. Compared with baseline values, the von Frey thresholds were significantly increased during the evaluation period in the dogs treated with morphine when the data were assessed as a group (**Figure 4**). However, when examined individually, the time for thresholds to decrease to values that were not significantly different from baseline was 2.8 ± 0.6 hours. In 1 dog, the von Frey threshold at the 3-hour time point was not significantly different from the baseline value but a threshold measurement was not obtained at the 4-hour time point because of a timing error. When the data for the morphine-treated dogs were assessed individually, none of the dogs had von Frey thresholds that were significantly greater than baseline values at 4 hours after treatment.

The limits of quantification for the plasma M6G and morphine assays were 25 and 5 ng/mL, respectively, as defined by the lowest point on the calibration curve within 20% of the actual value. The accuracy (deviation from actual value) and coefficient of variation were 8 ± 2% and 3 ± 1% for the morphine assay and were calculated from published equations.[24]

Morphine-6-glucuronide was not detected in any plasma sample. The mean ± SE plasma elimination half-life of morphine was 0.88 ± 0.13 hours (**Figure 5**). The calculated mean ± SE values for E_{MAX} and EC_{50} were 213 ± 43% and 13.92 ± 2.39 ng/mL, respectively (**Figure 6**).

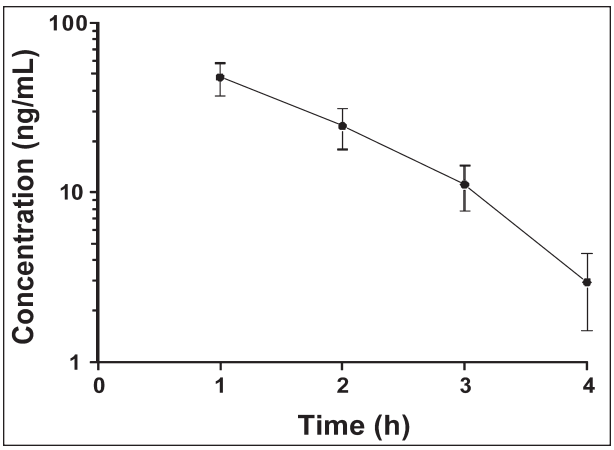

Figure 5—Mean ± SE plasma morphine concentration (log scale) in 6 dogs that received IV administration of a bolus of morphine sulfate (1 mg/kg; treatment phase of a crossover study) at 1, 2, 3, and 4 hours after treatment.

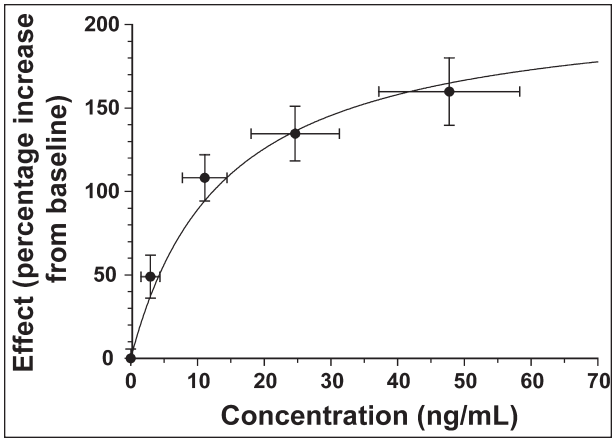

Figure 6—Pharmacodynamic model of morphine sulfate in 6 dogs that received IV administration of a bolus of the drug (1 mg/kg; treatment phase of a crossover study). The calculated values for the maximum effect or percentage change in von Frey threshold from baseline value and the concentration of morphine associated with a 50% maximal response were 213 ± 43% and 13.92 ± 2.39 ng/mL, respectively. Values are expressed as mean ± SE.

Discussion

Despite the common clinical and experimental administration of morphine to dogs, the dose response or the relationship between plasma morphine concentration and effect has never been objectively assessed to our knowledge. In 1 investigation,[25] morphine (0.5 mg/kg, unstated route of administration) administered to 2 dogs was associated with a 10% or greater increase in radiant heat thermal thresholds, compared with baseline values, over a 1-hour period. However, in that study, the sample size was small and statistical analysis of the data was not performed; moreover, baseline variability was not provided in that report. In another study,[26] the responses of dogs to 2 doses of morphine (0.25 and 0.5 mg/kg, IV) were determined by measuring changes in hind limb reflexes. Depression of the hind limb reflex was detected throughout the 5-hour experimental period of that study, and it was greater after administration of the higher dose of morphine. However, the experiments were performed in dogs with surgically severed spinal cords, and the relationship of depressed thermal reflexes to antinociception is unclear.

In dogs, morphine administered at 2 doses (0.5 mg/kg, IV, and 1 mg/kg, IM) did not result in significantly increased mechanical (pneumatic pressure) or thermal (incandescent bulb) thresholds, suggesting that either the dose-route combinations did not alter nociception or the data do not predict the antinociceptive effects of morphine.[1] The investigators suggested that the lack of response might be attributed to the study design, which precluded any pharmacokinetic-pharmacodynamic modeling.[1] Following IV administration of morphine (0.1 mg/kg) to dogs anesthetized by use of thiopental (4 mg/kg, IV to effect) and isoflurane inhalation (1.3%), a significantly decreased response to electrical stimulation of the tooth pulp (dental dolorimetry) was detected during a 60-minute test period, compared with findings in anesthetized control dogs that were treated IV with saline (0.9% NaCl) solution.[2] However, in that study, only 1 dose of morphine was evaluated, plasma concentrations of morphine were not measured, and anesthetic influences were likely to have contributed to the effects of morphine.

The effects of morphine have been assessed by use of the University of Melbourne pain scale in dogs that had undergone surgery and were receiving the drug via constant rate infusion or receiving morphine sulfate (1 mg/kg) administered IM every 4 hours.[11] No differences in pain scores between the treatment groups were found. However, 9 of the 10 categories evaluated by the pain scale (pupil diameter, heart rate, respiratory rate, rectal temperature, salivation, sedation, mental status, posture, and vocalization) are affected by pharmacologic effects of morphine that can be independent of the drug's antinociceptive effects. Additionally, no control (untreated) dogs were included in the study because of humane concerns. In the study reported here, the dogs salivated and appeared sedated for 7 to 12 hours following administration of morphine. Postures varied from apparently resting comfortably to sitting hunched in the back corner of the kennel, and all vocalized following the morphine injection. The incorporation of such variables into pain assessment scales used in studies to assess various doses of morphine administered via various routes makes it difficult to determine what dose of morphine is effective in dogs.[27-32]

One reason for the lack of reliable data regarding the antinociceptive activity of morphine in dogs is the lack of a reliable outcome measure of antinociception. The von Frey device used in our study was custom built for use in dogs and is commercially available.[b] Evaluation of the carpal pads provided the most consistent results in the control dogs. The metatarsal region as well as other locations were also assessed but resulted in higher baseline values, compared with values obtained from the carpal pads. The expected increase in von Frey thresholds was 150% to 200%; in the metatarsal region, such a percentage increase would have exceeded the load cell of the von Frey device. Additionally, the data obtained from the metatarsal region were associated with a higher SE value than that calculated for the carpal pad data; therefore, the carpal pads were assessed for all phases of the study. Tissue damage was not observed in any of the dogs during or after either phase. There was no evidence of hyperesthesia or tolerance to the stimulus in the control dogs. None of the dogs had signs of lameness at any time prior to, during, or after the study.

The von Frey device was technically simple to use and portable and caused no apparent tissue damage, which are characteristics desirable for assessment of antinociceptive effects in a laboratory or clinical setting. In the control dogs, there was no evidence of changes in thresholds due to tolerance, learned avoidance, or local hyperesthesia during an 8-hour evaluation period. Furthermore, in the treated dogs, the increased threshold values after morphine administration (compared with baseline values) indicate that the von Frey device was able to discriminate the antinociceptive effects of morphine. Additionally, sedation

(which interferes with subjective pain assessment scales) was detectable for as long as 12 hours in some treated dogs and outlasted the measured antinociceptive effects of morphine, suggesting the von Frey device is able to discriminate the antinociceptive effect from the sedative effect of morphine. Morphine-treated dogs were not evaluated for 8 hours, as were the control dogs, because all dogs had returned to baseline threshold values within 4 hours of treatment. The von Frey device used in the present study fulfils the criteria established by Beecher[33] for an ideal method of producing painful stimuli. As von Frey devices activate the same nerves transmitting naturally occurring pain, increases in von Frey thresholds from baseline values may be indicative of analgesia.[23] However, the level of increased thresholds that relate to clinically useful analgesia is not known.

In our study, the plasma concentration profile and elimination half-life (0.88 ± 0.13 hours) of morphine following IV administration of 1 dose (1 mg/kg [equivalent to 0.75 mg of morphine base/kg]) were similar to findings of previous studies[1,3,4,6,9,10] in dogs. The clearance and volume of distribution were not calculated because those values would have been overestimated since data from early time points representing the distribution phase were not collected. After morphine administration, M6G was not detected in any plasma sample from the 6 dogs at any time point (limit of quantification, 25 ng/mL). In humans, M6G is a major metabolite of morphine and may contribute as much as 66% of the antinociceptive effect following parenteral administration of the drug.[14-16] It is unlikely M6G contributes to the antinociceptive effects of morphine in dogs because of the low (undetectable) plasma concentrations detected in our study, which is consistent with results of other investgations.[8,13] Because M6G appears not to contribute to the antinociceptive effects of morphine in dogs, higher plasma concentrations of morphine may be required in this species than in humans to produce similar effects.

In humans, plasma morphine concentrations have been correlated with the drug's analgesic effect. In patients who have undergone surgery, the plasma concentrations of morphine reported to effectively achieve analgesia (as assessed via subjective scoring systems) range from 9.1 to 40 ng/mL.[16,34-38] In the dogs of the present study, the calculated value for the EC_{50} of morphine (13.92 ± 2.39 ng/mL) was similar to plasma concentrations associated with analgesia in people.

After IV administration of a bolus of morphine in dogs, there is a lag phase during which the drug diffuses into the CSF[9]; morphine also has a longer mean ± SE elimination half-life in CSF (121.0 ± 5.6 minutes), compared with that in plasma (65.4 ± 23.4 minutes).[9] Although CSF drug concentrations do not represent brain or spinal cord concentrations, those results may suggest a slower elimination of morphine from the CNS, compared with its elimination from plasma, indicating that plasma concentrations of morphine should be used only as a guide to effective concentrations unless steady state is reached.[39] Investigations in which morphine is administered as a constant rate infusion (to achieve steady state) to assess the pharmacodynamic effects during equilibrium between brain and plasma drug concentrations are warranted, and the von Frey device may allow such studies to be performed in dogs.

The von Frey device was well tolerated by the dogs of our study, and our data suggest that it can be used to assess the antinociceptive effects of morphine administered IV. By use of this device, pharmacodynamic modeling of a single dose of morphine in dogs was possible; results of the present study indicated that IV administration of morphine as a single bolus yields an EC_{50} of 13.92 ± 2.39 ng/mL in dogs. Further studies to assess the cumulative effects of morphine administered as a constant rate infusion or multiple doses are warranted.

a. Hill's d/d or Hill's maintenance, Hill's Pet Nutrition Inc, Topeka, Kan.
b. Model 2290-4 (modified), IITC Life Science, Woodland Hills, Calif.
c. Fast Steel, Polymeric Systems Inc, Phoenixville, Pa.
d. MLA, VistaLab Technologies, Mt Kisco, NY.
e. Morphine sulfate, Baxter Healthcare, Deerfield, Ill.
f. BD Vacutainer, Franklin Lakes, NJ.
g. Agilent 1100 series, Agilent Technologies, Wilmington, Del.
h. ESA, Coulochem II, Bedford, Mass.
i. Zorbax SB-phenyl, Agilent Technologies, Wilmington, Del.
j. Fischer Scientific, Fair Lawn, NJ.
k. HP ChemStation Rev A.06.03, Agilent Technologies, Wilmington, Del.
l. Varian C-8, Varian Inc, Palo Alto, Calif.
m. Lipomed Inc, Cambridge, Mass.
n. Sigma Stat 3.0, Systat Software Inc, Point Richmond, Calif.
o. WinNonlin, 4.0, Pharsight Corp, Mountain View, Calif.

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