Pharmacokinetics and pharmacodynamics of a constant rate infusion of fentanyl (5 µg/kg/h) in awake cats

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Objective—To evaluate the pharmacokinetics and thermal and mechanical antinociceptive effects of a fentanyl constant rate infusion (CRI) in conscious cats.

Animals—8 healthy adult cats.

Procedures—At a ≥ 14-day interval, 7 cats received a loading dose (LD) of fentanyl (5 µg/kg, IV [administered at 0 hours]) followed by fentanyl infusion (5 µg/kg/h, IV) for 2 hours or similar administrations of equivalent volumes of 0.9% saline (NaCl) solution. One cat received only the fentanyl treatment. For both treatments, sedation and adverse events were evaluated and mechanical threshold (MT) and thermal threshold (TT) testing was performed prior to (baseline) and at predetermined times up to 26 hours after LD administration; plasma fentanyl concentrations were determined at similar times when the cats received fentanyl.

Results—Fentanyl induced mild sedation during the infusion. The only adverse effect associated with fentanyl LD administration was profuse salivation (1 cat). Saline solution administration did not significantly change MT or TT over time. For the duration of the CRI, MT and TT differed significantly between treatments, except for TT 1 hour after LD administration. For the fentanyl treatment, MT and TT were significantly higher than baseline at 0.25 to 0.75 hours and at 0.25 to 1 hour, respectively. During the fentanyl CRI, mean ± SD plasma fentanyl concentration decreased from 4.41 ± 1.86 ng/mL to 2.99 ± 1.28 ng/mL and was correlated with antinociception; plasma concentrations < 1.33 ± 0.30 ng/mL were not associated with antinociception.


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µ-Opioid receptor agonists, such as fentanyl, are effective analgesics for severe pain. Fentanyl has a rapid onset and short duration of action and is typically administered intermittently as IV boluses or by IV infusion. In cats and dogs, it is used during both intra- and perioperative periods.1-5

Although fentanyl infusions have been used successfully in cats to supplement general anesthesia in a clinical setting,4,5 no reports are available on the pharmacokinetics and pharmacodynamics of fentanyl administered as a CRI in conscious cats, to our knowledge. The pharmacokinetics of fentanyl administered as a single IV injection or as an infusion in humans and dogs have been described.6-7 In humans, a low-dose CRI exceeding 2 hours resulted in fentanyl accumulation in peripheral tissue compartments.6 In contrast, plasma fentanyl concentration remained constant during a 4-hour infusion period (fentanyl dose rate, 10 µg/kg/h) in dogs and accumulation in peripheral tissue compartments did not occur.7 The pharmacokinetic characteristics of fentanyl after a single IV injection; single transdermal, intranasal, and oral administrations; and single applications in pluronic lecithin organogel to the inner aspect of the pinna have been previously studied in conscious cats and in cats anesthetized with isoflurane.10,11 In those studies in cats, fentanyl was rapidly distributed and eliminated. The minimum systemic fentanyl concentration necessary to provide analgesia is variable among individuals and different species. Robertson et al10 identified that a minimum plasma fentanyl

ABBREVIATIONS

| CRI | Constant rate infusion |
| LD | Loading dose |
| MS | Mass spectrometer |
| MT | Mechanical threshold |
| TT | Thermal threshold |
| UPLC | Ultra-performance liquid chromatography |
concentration of 1.07 ng/mL was required to provide antinociception (as determined by means of TT testing) in cats.

The objective of the study reported here was to evaluate the pharmacokinetics and thermal and mechanical antinociceptive effects of a 2-hour fentanyl CRI in conscious cats. Additionally, the behavioral effects associated with drug administration were recorded for assessment. The infusion dose rate selected for the investigation (5 µg of fentanyl/kg/h) was similar to a dose rate commonly used at our institution to provide analgesia during the perioperative period in cats undergoing surgery. We hypothesized that administration of a fentanyl CRI would increase MT and TT values and would not induce any behavioral adverse effects.

Materials and Methods

Animals—Eight healthy adult domestic shorthair cats (2 sexually intact females and 6 neutered males) were used in the study. Among the 8 cats, ages ranged from 2 to 6 years (mean ± SD age, 3.2 ± 1.3 years) and weight ranged from 3.7 to 6.7 kg (mean weight, 4.5 ± 1.0 kg). Cats were determined to be healthy on the basis of physical examination findings and results of a CBC and serum biochemical analysis (all clinicopathologic variables were within reference limits). Four weeks before the study began, the cats were tested for FIV and FeLV infections, and results were negative for all cats.

All cats were housed in a group in a climate-controlled room. Water was provided ad libitum, and dry and canned food was provided twice daily. All cats were socialized and familiarized with the threshold testing procedures and the testing environment for several weeks prior to study commencement. During the familiarization period, the MT and TT equipment was positioned on the cats and the observer (BA) became familiar with responses of individual cats to MT and TT testing. This work was approved by the University of Saskatchewan’s Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

Instrumentation and drug administration—On the day before use in an experiment, each participating cat was weighed and anesthetized with sevoflurane in oxygen delivered in an acrylic chamber. After anesthesia was induced, the trachea was intubated with a cuffed endotracheal tube, and anesthesia was maintained with sevoflurane in oxygen delivered via a Bain circuit. Hair on the lateral aspects of the thorax, around the entire circumference of the lower portion of the thoracic limbs, and on the ventral aspect of the neck was clipped. A 4-F catheter was inserted aseptically in the entire circumference of the lower portion of the circuit. Hair on the lateral aspects of the thorax, around the external auditory meatus, and on the lateral aspects of the thorax was trimmed with sevoflurane in oxygen delivered via a Bain cuffed endotracheal tube, and anesthesia was maintained with sevoflurane in oxygen delivered in an acrylic chamber. After anesthesia was induced, the trachea was intubated with a cuffed endotracheal tube, and anesthesia was main-
limbs with a wrapping of hook and loop material. For the duration of cephalic catheter placement, the contralateral thoracic limb was used for MT testing; after the 2-hour infusion period, application of the testing system actuator was alternated between thoracic limbs every 2 hours. The actuator held 3 retractable, well-rounded pins (each tipped with a 2.4-mm-diameter ball bearing) in a 10-mm triangular pattern. The actuator was driven pneumatically by an air-filled syringe. When the pressure within the actuator was increased, the pins were advanced against the cranialateral aspect of the antebrachium. For each test, the actuator was connected via 150-cm-long nonexpandable polyethylene tubing to a 20-mL syringe filled with air and a pressure transducer. The syringe plunger was depressed manually, applying increasing force at a rate of 0.8 N/s. The manual increase in pressure was controlled by use of 2 warning lights, which indicated that the rate was too low or too high, respectively. If the neither warning light was activated during application of the stimulus, the operator was assured that the force did not increase or decrease by >1 N. Mechanical threshold was recorded when the cat withdrew, raised, or shook its limb; jumped forward; or turned toward or tried to bite the actuator. When the cat reacted, the pressure was immediately released by detaching the 20-mL syringe, and the force was recorded as the MT at that time point. A safety cutoff value was set at 20 N to prevent tissue damage if the cat did not react before this force was reached.13

For measurement of TT, a wireless testing system6 was used. The thermal stimulus was provided by a small probe that contained both a heating element and a temperature sensor. The probe was held in place against the shaved region of the cat's thorax by an elastic and hook and loop material band. A pressure blader was connected via 150-cm-long nonexpandable polyethylene tubing to a 20-mL syringe filled with air and a temperature sensor. The probe was held in place against the shaved region of the cat's thorax by an elastic and hook and loop material band. A pressure blader was inflated to a known pressure, ensuring that the thermal probe was held against the skin with a repeatable and constant force. The heater was activated via a handheld infrared remote control.

Before each testing, skin temperature was measured. When activated, the probe heated at a rate of 0.6°C/s with a safety cut-off value of 33°C to prevent skin damage.12 During each measurement, the stimulus was terminated when the cat responded by jumping, flinching, turning toward the probe, licking or biting the probe area, or when the cutoff temperature was reached. The temperature at the termination of the stimulus was recorded as the TT at that time point.

Blood sample collection and drug analysis—Blood samples were withdrawn via the jugular catheter. The volume (1.2 to 1.6 mL/sample) of blood collected was adjusted so that <10% of each cat's estimated total blood volume (67 mL/kg)10 was removed during the 24-hour experimental period. An equal volume of saline solution was injected after each blood sample was withdrawn. Blood samples were immediately transferred into tubes containing lithium heparin and centrifuged (2,000 × g) for 10 minutes. Plasma was separated from each sample and stored at −20°C for a maximum of 4 weeks before the fentanyl concentration assay was performed.

Plasma samples were analyzed with a validated UPLC method with tandem MS detection (UPLC-MS/MS). One hundred microliters of each plasma sample was precipitated with 200 μL of methanol containing fentanyl D5 in microcentrifuge tubes. The tubes were then vortexed vigorously for 30 seconds. After thorough mixing, the tubes were centrifuged for 5 minutes. The clear supernatant from each microcentrifuge tube was then added directly to wells in a 96-well plate for injection onto the UPLC-tandem MS (injection volume, 10 μL). Chromatography was performed with a C18 column (4.6 × 50 mm; 1.8 μm particle size) with the column compartment set at 30°C. A mobile-phase gradient (from 90% aqueous to 90% organic) was incorporated with a 98% organic flush before the next injection and consisted of water with 0.1% formic acid (aqueous phase) and acetonitrile with 0.1% formic acid (organic phase), delivered at a flow rate of 0.6 mL/min. The MS was used in multiple reaction monitoring mode in positive electrospray mode. The m/z transitions were monitored at transitions 337.1 → 187.8 (precursor/product pair) for fentanyl and 342.1 → 187.8 (precursor/product pair) for fentanyl D5. Chromatograms were evaluated with an internal standard method, with calculation of peak-area ratios of fentanyl to fentanyl D5. A 9-point calibration curve (0, 0.05, 0.10, 0.20, 0.50, 1.0, 2.5, 5.0, and 10.0 ng/mL) was constructed, and linear coefficients of determination (R2 = 0.999) were obtained for all analysis runs. Fentanyl extraction efficiency was between 90% and 110%, and the limit of detection was 0.01 ng/mL.

Pharmacokinetic analysis—Noncompartmental methods were used for assessment of pharmacokinetic parameters of fentanyl in cats. Values of the area under the plasma concentration versus time curve from time 0 to infinity were calculated by the trapezoidal rule-extrapolation method. The elimination rate constant β (representing the rapidly equilibrating peripheral compartment) was determined by linear regression analysis of the postdistributive log-linear terminal serum concentration-time determinations. A very slow log terminal phase (γ), attributable to a slowly equilibrating peripheral compartment, was observed but not used in the calculation of the pharmacokinetic parameter estimates. A software program was used for pharmacokinetic parameter estimation of individual cat concentration versus time data.

Statistical analysis—A commercially available software package was used for statistical analyses. Descriptive statistics were computed to confirm that values of each measured variable (skin temperature, MTs, and TTs) were normally distributed. Baseline MT and TT for each treatment were compared with a 1-way ANOVA. Skin temperature, MT, and TT at each time point for each treatment were compared by use of a 2-way ANOVA for repeated measures, followed by a Bonferroni adjustment. The within-treatment changes were examined with a 1-way ANOVA for repeated measures with a post hoc Dunnett multiple comparison test against baseline. All data are reported as mean ± SD. A value of P ≤ 0.05 was considered significant.
Results

Because of the necessity for a surgical cut down for the jugular catheter placement, 1 cat received only treatment with fentanyl. Thus, data for all variables of interest for both treatments were obtained from 7 cats for each treatment; however, data for the pharmacokinetic analyses were available for 8 cats.

Skin temperature did not vary significantly \((P > 0.05)\) with time for either treatment. No difference in baseline MT or TT was detected between treatments. The MT and TT were significantly altered from baseline during the fentanyl CRI (Figure 1). Mean ± SD MT was significantly higher than baseline \((5.3 ± 0.8 \text{ N})\) at 0.25 (peak, \(11.8 ± 2.9 \text{ N}\)), 0.75, and 1 hour. Thermal threshold was significantly higher than baseline \((44.2 ± 0.3°C)\) at 0.25 (peak, \(50.0 ± 2.9°C\)) and 0.75 hours. However, when cats received treatment with saline solution, MT and TT did not change significantly over time. For the duration of the 2-hour CRI, MT and TT differed significantly between treatments at each time point, except for TT at 1 hour after LD administration.

During the 2-hour CRI, plasma fentanyl concentration decreased from \(4.41 ± 1.86 \text{ ng/mL}\) to \(2.99 ± 1.28 \text{ ng/mL}\) and was associated with antinociception (Figure 2). Plasma fentanyl concentrations < \(1.33 ± 0.30 \text{ ng/mL}\) (2.25 hours after LD administration) were not associated with antinociception.

With cessation of the infusion, plasma concentrations of fentanyl decreased quickly with a mean elimination half-life of 2.4 hours. Mean systemic clearance was \(1.37 ± 0.32 \text{ L/h/kg}\), and the mean apparent volume of distribution was \(4.42 ± 1.41 \text{ L/kg}\) (Table 1).

No serious adverse effects were associated with either treatment; 1 cat salivated profusely for 7 minutes after administration of the fentanyl LD. Mydriasis developed within minutes after administration of the fentanyl LD in all cats and persisted for the duration of the infusion. When cats were treated with fentanyl, most became sedated (sedation score, 1); 3 cats were sedated during the first hour of the 2-hour infusion, and 3 cats were sedated for the entire infusion period. Locomotor activity decreased in all cats, and most cats remained in a sternal sitting position during the infusion. All cats were easy to handle during the experiments; 4 cats had mild signs of euphoria including increased rubbing and purring when interacting with humans and kneading with their forepaws.

Discussion

Results of the present study indicated that administration of an LD of fentanyl \((5 \mu g/kg)\) followed by IV CRI of fentanyl \((5 \mu g/kg/h)\) for 2 hours induced thermal and mechanical antinociception in awake cats. In this study, we also characterized the pharmacokinetic effects of the fentanyl treatment in cats. However, the pharmacokinetic data should be interpreted with respect to its few limitations. The pharmacokinetic analysis might have been adversely affected by the choice of time points at which data were collected. The selected time points in the early part of the experiment did not include the initial distribution phase of the fentanyl LD, and more sample collections should have been performed during the first 15 minutes of the infusion to accurately define this pharmacokinetic phase. In the present study, the lower plasma concentra-
After cessation of the CRI, the fentanyl plasma concentration decreased rapidly during the first 30 minutes and more gradually thereafter. In humans, plasma fentanyl concentration rapidly continues to increase once the duration of fentanyl infusion exceeds 2 hours; however, the fentanyl CRI investigated in the present study was not longer than 2 hours and we cannot speculate on whether accumulation would have occurred in the study cats as a result of prolonged infusion. At the termination of the brief fentanyl infusion in the present study, it is likely that distribution equilibrium in all peripheral compartments was not achieved. Continual redistribution into these compartments in addition to the elimination of fentanyl likely contributed to the rapid decrease in central compartment drug concentrations. In the present study, no sustained antinociceptive effects following discontinuation of the fentanyl CRI were observed, which suggested that fentanyl concentrations at the effect site rapidly decreased at termination of the infusion. However, in that other study, blood samples were collected from anesthetized humans and anesthesia-associated alterations in blood flow and hepatic metabolism might have affected plasma fentanyl concentrations and promoted drug accumulation. In awake dogs administered an LD of 10 µg/kg of fentanyl followed by a fentanyl infusion at a rate of 10 µg/kg/h, plasma fentanyl concentrations remain relatively constant during various infusion periods (1, 3, and 4 hours). The duration of administration influenced total body clearance of fentanyl and a gradual increase in plasma concentrations over the infusion period was observed. After 1- to 4-hour infusions, the end-infusion plasma fentanyl concentrations in those dogs ranged from 1.01 to 1.25 ng/mL which is considerably lower than the end-infusion concentration in the cats of the present study (2.99 ng/mL). The steady-state volume of distribution in the cats of the present was 1.37 L/kg, compared with 2.87 to 3.71 L/kg in the dogs. Interspecies differences in hepatic metabolism, which is the principal determinant of opioid clearance, might explain the slower fentanyl clearance in cats, compared with that in dogs. The high total body clearance in dogs might also indicate that fentanyl may be metabolized in extrahepatic sites and eliminated by extrarenal routes. Extrarenal metabolism of fentanyl in cats has not been reported, to our knowledge.

Sano et al speculated that analgesia might be sustained for longer periods after discontinuation of fentanyl infusions administered for > 3 hours in dogs because total body clearance decreased with increasing duration of infusion; however, no antinociceptive testing was performed in that study. In the cats in the present study, antinociceptive effects were not sustained and appeared to correlate with plasma fentanyl concentration. This close relationship between the time course of antinociception and plasma fentanyl concentration

Table 1—Pharmacokinetic parameters for fentanyl determined in 8 conscious cats that received an LD of fentanyl (5 µg/kg, IV [administered at 0 hours]) followed by a CRI of fentanyl (5 µg/kg/h, IV) for 2 hours.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC∞</td>
<td>11.7 ± 3.64</td>
</tr>
<tr>
<td>AUMC∞</td>
<td>36.3 ± 9.1</td>
</tr>
<tr>
<td>Cl (L/h/kg)</td>
<td>1.37 ± 0.32</td>
</tr>
<tr>
<td>Vss (L/kg)</td>
<td>4.42 ± 1.41</td>
</tr>
<tr>
<td>t1/2γ</td>
<td>2.37 ± 0.37</td>
</tr>
<tr>
<td>t1/2β</td>
<td>8.97 ± 1.55</td>
</tr>
</tbody>
</table>

A blood sample was collected for analysis from each cat at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.25, 2.5, 2.75, 3, 4, 6, 8, 10, 14, and 26 hours after LD administration.

AUC∞ = Area under the plasma fentanyl curve extrapolated to infinity. AUMC∞ = Area under the first moment curve extrapolated to infinity. Cl = Systemic plasma clearance. t1/2γ = Half-life of β phase. t1/2β = Half-life of γ phase. Vss = Apparent volume of distribution at steady state.

Figure 2—Mean ± SD plasma fentanyl concentrations in 8 conscious cats that received an LD of fentanyl (5 µg/kg, IV [administered at 0 hours]) followed by a CRI of fentanyl (5 µg/kg/h, IV) for 2 hours. A blood sample was collected for analysis from each cat at predetermined times up to 26 hours after LD administration.
in the present study suggested minimal hysteresis, but the correlation of effect and plasma drug concentration is complex and further studies with infusions at different dose rates and for different durations are required.

Adverse behavioral effects are a concern when opioids are used in cats. In the present study, cats remained calm; most became sedated after receiving the LD of fentanyl and remained sedated for most of the infusion period. These findings are in contrast to those of a previous study in cats in which the behavioral effects of multiple doses of morphine and fentanyl were investigated. In that study, 2 of 6 cats had increased locomotor activity for 5 minutes and appeared dysphoric after IV administration of a dose of 10 µg of fentanyl/kg. Behavior in the cats in the present study was more comparable to the behavior of those other cats after treatment with morphine; the cats’ activity level decreased, and they remained in a sternal sitting position with a fixed stare but appeared euphoric while interacting with people. This difference in cats’ behavioral response to opioids in the 2 studies could be explained by pharmacogenetic variation in morphology and sequencing of opioid receptors. Further research of the genetic variation in opioid receptors among individual cats is required to determine how variations in morphology and sequencing of opioid receptors might influence the analgesic efficacy of or adverse reactions to opioids. In the present study, no adverse behavioral effects in the cats were observed, but the cats were healthy and apparently pain free. In a clinical setting, cats that are ill or have signs of pain may have different behavioral responses following administration of fentanyl.

Results of the present study indicated that administration of 5 µg of fentanyl/kg IV followed by a CRI of fentanyl at a rate of 5 µg/kg/h for 2 hours was well tolerated by awake cats and that thermal and mechanical antinoception was induced during the infusion period. However, further studies involving different infusion rates and longer infusion periods are warranted to provide better understanding of the pharmacokinetics of fentanyl in cats.

References