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RESEARCH PAPER

Effective plasma alfaxalone concentration to produce immobility in male neutered cats

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Abstract

Objective To determine the effective plasma alfaxalone concentration for the production of immobility in cats.

Study design Prospective up-and-down study.

Animals Sixteen 1–2 year old male castrated research cats.

Methods Cats were instrumented with catheters in a jugular and a medial saphenous vein. Alfaxalone was administered via the medial saphenous catheter, using a target-controlled infusion system. The infusion lasted for approximately 32 minutes. A noxious stimulus (tail clamp) was applied 30 minutes after starting the alfaxalone infusion, until the cat moved or 60 seconds had elapsed, whichever occurred first. The target alfaxalone concentration was set at 5 mg L^{-1} in the first cat and increased or decreased by 1 mg L^{-1} in subsequent cats, if the previous cat had moved or not moved in response to stimulation, respectively. This was continued until six independent crossovers (different responses in pairs of subsequent cats) had been observed. Blood samples were collected before alfaxalone administration, and 15 and 31 minutes after starting the administration, for the determination of plasma alfaxalone concentration using liquid chromatography/tandem mass spectrometry. The alfaxalone concentration yielding a probability of immobility in 50% (EC_{50}), 95% (EC_{95}) and 99% (EC_{99}) of the population, and their respective 95% Wald confidence intervals were calculated.

Results The EC_{50}, EC_{95} and EC_{99} for alfaxalone-induced immobility were 3.7 (2.4–4.9), 6.2 (4.7–) and 7.6 (5.5–) mg L^{-1}, respectively.

Conclusions and clinical relevance The effective plasma alfaxalone concentration for immobility in cats was determined. This value will help in the design of pharmacokinetic-based dosing regimens.

Keywords alfaxalone, cats, pharmacology.

Introduction

Alfaxalone is an anesthetic neurosteroid. It is currently commercially available as a solution containing alfaxalone solubilized in hydroxypropyl-β-cyclodextrin, and is labeled in the United States, Canada, Europe, Korea, Japan, New Zealand, South Africa and Australia for induction and maintenance of anesthesia via intermittent intravenous (IV) boluses in dogs and cats. The pharmacokinetics of alfaxalone in cats have been characterized (Whittem et al. 2008); however, to the authors' knowledge, the effective plasma concentration of alfaxalone has not been reported in either dogs or cats, making it difficult to use the available pharmacokinetic information to calculate adequate dosing for induction or maintenance of anesthesia, by intermittent IV boluses or IV infusion. The objective of this study was to estimate the effective plasma alfaxalone concentration in 50%, 95% and 99% of the cat population, resulting in immobility following noxious stimulation.

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Materials and methods

This study was approved by the Institutional Animal Care and Use Committee at the University of California, Davis. Healthy, 1−2 years old, male, neutered cats were used. The health status of each cat was assessed by history and complete physical examination 2 days before, as well as on the day of the experiment. In addition, cats were observed daily for any signs of disease for 7 days before the experiment was initiated. Husbandry conditions for this institution have been previously described (Honkavaara et al. 2017). The cats were fed Laboratory Feline Diet 5003 (LabDiet, MO, USA) once daily.

On the day prior to the experiment, each cat was briefly anesthetized with isoflurane in oxygen. The skin over a jugular vein and a medial saphenous vein was clipped and aseptically prepared using chlorhexidine and alcohol. A 20 gauge, 48 mm or a 19 gauge, 150 mm catheter was inserted in the jugular vein for collection of blood samples. A 20 gauge, 48 mm catheter was inserted in the medial saphenous vein for drug administration. Infusion plugs were placed on the catheters, and the catheters were flushed with heparinized 0.9% weight/volume (w/v) saline solution and secured to the skin with tape (medial saphenous) or suture (jugular). A light bandage was placed over the catheters and cats were allowed to recover from anesthesia. An Elizabethan collar was placed around the neck and cats were returned to their room.

On the day of the experiment, the cat's body weight was measured. A physical examination was performed, including the measurement of pulse rate (PR), respiratory rate (f_R) and rectal body temperature (RT). The patency of the catheters was verified by injecting a small volume of heparinized saline solution. Alfaxalone (10 mg mL^{-1}) in hydroxypropyl-β-cyclodextrin was administered via the medial saphenous catheter, using a target-controlled infusion system. An alfaxalone preserved formulation was administered in the study. The alfaxalone formulation contained the preservatives ethanol (150 mg mL^{-1}), chlorocresol (1 mg mL^{-1}) and benzethonium chloride (0.2 mg mL^{-1}). The target-controlled infusion system consisted of a syringe pump (PHD2000; Harvard Apparatus, MA, USA) and computer program (Rugloop I; Demed, Belgium). The system rapidly loaded the central compartment to the target concentration by delivering a bolus calculated from $[T] \times V_c$, and maintained this concentration via a variable infusion rate updated every 10 seconds, according to the following equation: $R = [T] \times V_c \times (k_{10} + k_{12} \times e^{-k21 \times t})$, where R is the infusion rate; [T] is the target plasma concentration; V_c is the volume of the central compartment; k_{10}, k_{12} and k_{21} are microrate constants and t is the infusion time. V_c, k_{10}, k_{12} and k_{21} were 302 mL kg^{-1}, 0.101 $minute^{-1}$, 0.323 $minute^{-1}$ and 0.066 $minute^{-1}$, respectively. The latter values were obtained from the best fitting pharmacokinetic model obtained from 24 cats following IV bolus administration of the same alfaxalone formulation (RD0327) in a different study (Pasloske et al. 2018).

Observations were made on whether any cat exhibited signs of pain or discomfort in response to the injection of the preserved alfaxalone formulation. The cat was placed on a heating pad. PR (palpation of the femoral pulse over 15 seconds), f_R (observation of chest excursions over 15 seconds) and RT (electronic thermometer) were measured 15, 25 and 45 minutes after starting the drug administration. A noxious stimulus (clamping of the tail using a 20 cm Martin forceps closed to the first ratchet) was applied 30 minutes after starting the target-controlled infusion. The stimulus was continued until 1 minute had elapsed, or until movement was observed, whichever occurred first, and the response was recorded as positive (movement) or negative (lack of movement). Any movement of the limbs or head, except related to swallowing or coughing, was considered positive.

Blood samples (2 mL) were collected prior to drug administration, 15 minutes after starting drug administration, and immediately after the noxious stimulus was delivered. Prior to sampling, blood (3 mL) was aspirated into a syringe containing a small amount of heparinized saline and later returned after the actual sample had been collected. Blood was transferred to tubes containing lithium heparin, placed on ice and centrifuged at 3901 ***g*** and 4 °C for 10 minutes within 60 minutes of collection. The plasma was separated, transferred to cryotubes and frozen at −80 °C until analyzed for alfaxalone concentration.

Drug administration was discontinued following collection of the last blood sample, and the cat was allowed to recover under observation. Times from discontinuation of drug administration to head lift, return to sternal recumbency and ability to stand without assistance were recorded. The quality of anesthesia induction, the quality of anesthesia and the quality of recovery from anesthesia were subjectively assessed using a 100 mm visual analog scale (VAS). For quality of induction, 0 was defined as

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