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RESEARCH PAPER

Anaesthetic induction with alfaxalone in the ball python (*Python regius*): dose response and effect of injection site

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Abstract

Objective To characterise the minimum dose of intramuscular alfaxalone required to facilitate intubation for mechanical ventilation, and to investigate the impact of cranial versus caudal injection on anaesthetic depth.

Study design Randomised crossover study.

Animals Six healthy juvenile ball pythons (*Python regius*).

Methods Three dosages (10, 20 and 30 mg kg^{-1}) of alfaxalone were administered to each python in a caudal location with a minimum 2 weeks washout. Induction and recovery were monitored by assessing muscle tone, righting reflex, response to a noxious stimulus and the ability to intubate. A subsequent experiment assessed the influence of injection site by comparing administration of 20 mg kg^{-1} alfaxalone in a cranial location (1 cm cranial to the heart) with the caudal site. Respiration rate was monitored throughout, and when intubation was possible, snakes were mechanically ventilated.

Results Regardless of dose and injection site, maximum effect was reached within 10.0 ± 2.7 minutes. When administered at the caudal injection site, intubation was only successful after a dosage of 30 mg kg^{-1}, which is higher than in previous reports for other reptiles. However, intubation was possible in all cases after 7.2 ± 1.6 minutes upon cranial administration of 20 mg kg^{-1}, and anaesthetic duration was significantly lengthened ($p < 0.001$). Both 30 mg kg^{-1} at the caudal site and 20 mg kg^{-1} at the cranial site led to apnoea approximately 10 minutes post-injection, at which time the snakes were intubated and mechanically ventilated.

Conclusions and clinical relevance Alfaxalone provided rapid, smooth induction when administered intramuscularly to pythons, and may serve as a useful induction agent prior to provision of volatile anaesthetics. The same dosage injected in the cranial site led to deeper anaesthesia than when injected caudally, suggesting that shunting to the liver and first-pass metabolism of alfaxalone occur when injected caudally, via the renal portal system.

Keywords alfaxalone, gamma-aminobutyric acid-$_A$ agonist, injectable anaesthesia, injection site, reptile, snake.

Introduction

General anaesthesia of snakes is commonly performed using inhalant agents (Mosley 2005; Bertelsen 2014). Current recommendations for the use of volatile anaesthetics state that the snake is induced using either a chamber, tube, mask or bag before intubation for ventilation with the same agent (Bertelsen 2014). However, *Python regius* experiences metabolic acidosis under isoflurane anaesthesia, particularly following induction (Jakobsen et al. 2017). Injectable anaesthetics provide an interesting alternative to alleviate such detrimental physiological consequences and stress during induction. Propofol, a commonly used injectable agent, provides fast and reliable induction in snakes and other reptile species (Bertelsen 2014), but requires vascular access, which is challenging in many

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snakes. Intramuscular administration of alpha-2-adrenergic agonists such as medetomidine and dexmedetomidine combined with ketamine has been used for reptile anaesthesia (Holz & Holz 1994; Greer et al. 2001; Heaton-Jones et al. 2002). However, dissociative anaesthetics are associated with long recovery times and variations in anaesthetic depth (Bertelsen 2014). Therefore, alternative agents should also be explored.

Alfaxalone (Alfaxan; 3α-hydroxy-5α-pregnane-11,20-dione) provides muscle relaxation and anaesthesia in mammals (Rupprecht & Holsboer 1999), and can be administered intramuscularly without irritation of the tissue in rats (Bennett 1991). While alfaxalone is currently only registered for use in cats and dogs (Zaki et al. 2009), innocuous anaesthetic induction has been reported for several reptile species such as red-eared slider turtles (*Trachemys scripta elegans*; Kischinovsky et al. 2013), horsfield tortoises (*Agrionemys horsfieldii*; Hansen & Bertelsen 2013) and green iguanas (*Iguana iguana*; Bertelsen & Sauer 2011). It is a synthetic neuroactive steroid that enhances gamma-aminobutyric acid-$_A$ binding, via positive allosteric modulation of endogenous receptors in the central nervous system (Rupprecht & Holsboer 1999; Belelli & Lambert 2005).

Alfaxalone is metabolised in the liver in mammals (Sear & McGivan 1981; Warne et al. 2015), which is also likely to be the case in reptiles. However, reptiles have a renal portal system that drains the caudal blood towards the liver (Holz et al. 1997b), therefore, injection of alfaxalone to this region of the body may render it less bioavailable and increase dose requirements compared with more cranial injections. In fact, other drugs that undergo hepatic metabolism, such as tramadol and medetomidine, achieve lower plasma concentrations or exhibit reduced efficacy when injected caudally compared with cranially in reptiles (Olsson & Phalen 2012; Giorgi et al. 2015). Caudal injection is, nevertheless, preferred for a variety of reasons in certain reptiles, particularly in dangerous species (Olsson & Phalen 2012), and when restraining snakes using tubes (Sladky & Mans 2012). Therefore, it is pertinent to study the potential influence of injection site on anaesthetic efficacy, as dose requirements may be reduced at more cranial sites.

This study aimed to determine an appropriate intramuscular induction dosage of alfaxalone to facilitate intubation prior to surgical anaesthesia in the ball python (*P. regius*). The effect of three different dosages (10, 20 and 30 mg kg^{-1}) on skeletal muscle tone, response to stimulation, respiration and induction and recovery times was assessed. To study the impact of first-pass hepatic metabolism, the influence of the middle dosage (20 mg kg^{-1}) was assessed at two injection sites.

Materials and methods

This study was conducted with permission from the Danish Animal Experiments Inspectorate (Permit Number 2012-15-2934-00280).

Animals

A total of six juvenile pythons (*P. regius*) of both sexes (four females and two males) were individually housed in a temperature- and humidity-controlled environment (26−28 °C with access to a heat source, 70−80% humidity), at a 12:12 hour light−dark cycle. Snakes were fed an appropriately sized dead rodent on a weekly basis and fasted for 7 days prior to each experimental round. Animals were only used if deemed healthy by brief clinical assessment prior to each trial.

The group sample size was determined based on a power calculation conducted following a pilot study, where two pythons were administered 10, 20 and 30 mg kg^{-1} intramuscular alfaxalone in a randomised, crossover design. The mean total anaesthetic duration was used to calculate a desired sample size based on a power (β) of 0.8 and type 1 error rate (α) of 0.05. We expected significant differences between the 20 and 30 mg kg^{-1} dosages and 10 and 30 mg kg^{-1} dosages. When comparing the 20 and 30 mg kg^{-1} dosages, the mean anaesthetic durations were 24 and 60 minutes, respectively; with a pooled standard deviation of 22, the *n* returned was six. We also compared the 10 and 30 mg kg^{-1} dosages (mean durations 20 and 60 minutes, respectively; pooled standard deviation of 23), which also returned a desired sample size of six.

Induction with alfaxalone

This experiment followed a randomised, crossover design, in which each dosage was administered to each snake in a random order. The dosage was decided at the start of each trial by random number generation. There was a minimum 2 weeks washout period between trials, and snakes were not used while shedding. The pythons were manually restrained and 10, 20 or 30 mg kg^{-1} alfaxalone (Alfaxan; Jurox, UK) was administered into the epaxial muscle in the posterior third of the body, using a 23 gauge needle

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