

Clinical measurements performed during alfaxalone total intravenous anaesthesia (TIVA) for radiography and neurophysiological investigations in dogs.

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Alfaxalone CRI in dogs

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This study was supported by the BBSRC grant number BB/L00240X/1. The alfaxalone was supplied by Jurox (UK) Limited. JRH, HJ, and MG positions were funded by the BBSRC grant number BB/L00240X/1. The authors declare no other conflict of interest.

## SHORT COMMUNICATION

### **Clinical measurements performed during alfaxalone total intravenous anaesthesia (TIVA) for radiography and neurophysiological investigations in dogs**

#### **Abstract (289 words)**

**Objective** To describe clinically relevant, physiological measurements collected during a 3-hour duration alfaxalone total intravenous anaesthesia.

**Study design** Case series.

**Animals** A total of 112 client-owned middle aged or older dogs.

**Methods** Dogs were premedicated with intramuscular acepromazine (0.03 mg kg<sup>-1</sup>).

Anaesthesia was induced and subsequently maintained for up to 3 hours with alfaxalone administered intravenously. Dogs breathed 100% oxygen via an endotracheal tube. Heart rate, respiratory rate, and blood pressure were evaluated 30 minutes after administration of acepromazine and used as baseline values for comparisons of intra-anaesthetic data. Blood glucose was measured one week prior to anaesthesia and every hour during alfaxalone anaesthesia. Quality and duration of recovery were recorded. Mean data for physiological variables were compared over three time points; before induction of anaesthesia, for the first hour of anaesthesia and from 60 minutes to discontinuation of anaesthesia.

**Results** Mean induction dose of alfaxalone was 1.4 (95% CI 1.3 - 1.5) mg kg<sup>-1</sup>. Post induction apnoea for greater than 60 seconds occurred in 13 (11.6%) dogs. Mean alfaxalone infusion rate during the first 60 minutes of anaesthesia was 0.099 mg kg<sup>-1</sup> minute<sup>-1</sup>; from 60 minutes until discontinuation of anaesthesia, mean infusion rate was 0.092 mg kg<sup>-1</sup> minute<sup>-1</sup>.

Heart rate was well maintained; hypotension (mean arterial blood pressure < 60 mmHg) was encountered in 23 (21%) of dogs. Blood glucose levels did not alter during anaesthesia.

Median time between discontinuation of alfaxalone infusion and extubation was 17 (7 - 35

minutes), time to assuming sternal recumbency was 75 (58 - 110 minutes), and time to standing was 109 (88 - 140 minutes).

**Conclusions and clinical relevance.** Alfaxalone infusion provided effective anaesthesia in this population. In a minority of cases respiratory and haemodynamic support of the patient was required.

**Keywords** Alfaxalone, Dog, Total Intravenous Anaesthesia

## **Introduction**

Alfaxalone solubilised in 2- hydroxypropyl- $\beta$ -cyclodextrin (HPCD) is licensed for use in dogs for induction and maintenance of anaesthesia by Continuous Rate Infusion (CRI). The use of alfaxalone as an intravenous induction agent has not demonstrated any significant effects on blood glucose concentration (Muñoz et al. 2017), and HPCD is reported to be largely renally excreted, without undergoing significant amylase catalysed degradation to glucose (Irie & Uekama 1997). However, it is possible that exposure to the carrier for some hours during total intravenous anaesthesia could produce alteration in serum glucose concentrations. The effect of a CRI of alfaxalone in HPCD on serum glucose measurements has not knowingly been previously reported in dogs.

The objectives of the study were as follows: We aimed to report routinely measured clinical physiological parameters during the anaesthetic episode, and to measure glucose concentrations hourly during the alfaxalone infusion. We hypothesised that the qualities of anaesthesia in this population of dogs would reflect previously described findings of hypoventilation and mild haemodynamic changes (Ambros et al. 2008; Suarez et al. 2012; Herbert et al. 2013), and that alfaxalone infusion would have no significant effect on serum glucose concentrations.

## **Materials and methods**

### **Ethics**

The study was conducted under the terms of the Animal (Scientific Procedures) Act, 1986 (as amended, 2013).

### **Recruitment criteria**

Dogs with osteoarthritis and a healthy control group were recruited to the study. Dogs were healthy (American Society of Anesthesiologists grade I/II) on the basis of preanaesthetic physical examination and routine biochemistry and haematology prior to anaesthesia. For the osteoarthritis group suitable dogs were 12 kg bodyweight and over, of any age, body condition and sex exhibiting suspected painful uni- or bilateral coxofemoral or stifle degenerative joint disease (DJD) as evidenced by lameness/stiffness/difficulty rising or ascending steps. Dogs with primarily forelimb lameness were excluded. For the control group, dogs were 6 years old or greater and 12 kg bodyweight and over, exhibiting no evidence of lameness or stiffness and with no other painful condition (e.g. otitis externa) and no previous diagnosis of OA. We aimed to recruit 100 dogs with OA and 35 control dogs based on a sample size calculation associated with the primary reason to perform the study (i.e. measurement of nociceptive withdrawal reflexes in dogs).

### **Study protocol**

Owners of eligible dogs were asked to attend with their dog for a screening appointment, at which signed consent to participate was obtained. Jugular vein blood samples were collected and submitted for biochemistry and haematology, and whole blood glucose measurement was

performed using a handheld device (Alphatrak 2; Abbott Diabetes Care Inc., CA, USA) validated for measurement of glucose concentrations in canine blood.

#### Premedication and preanaesthetic measurements

Seven days after the screening appointment, dogs were admitted in order to undergo radiography and Nociceptive Withdrawal Reflex (NWR) testing under general anaesthesia. Food was withheld for 8 hours prior to anaesthesia. Acepromazine ( $0.03\text{mg kg}^{-1}$ ) (ACP  $2\text{mg mL}^{-1}$  solution; Elanco Animal Health, Basingstoke, UK) was administered into the cervical epaxial muscles, and dogs were left undisturbed for 30 minutes, following which a cephalic venous catheter was placed. Indirect oscillometric (Datex-Ohmeda S5) systolic (S), diastolic (D), and mean (M) arterial pressure (AP) was determined using a cuff width of 30-40% limb circumference with the dog in lateral recumbency, using the dorsal pedal arterial site. Heart (HR) and respiratory rate ( $f_r$ ) were assessed manually and recorded prior to induction of anaesthesia.

#### Anaesthesia

Alfaxalone [Alfaxan, Jurox (UK) Ltd, Crawley, UK] ( $1\text{-}2\text{ mg kg}^{-1}$ ) was administered intravenously over 60 seconds until orotracheal intubation was possible. Oxygen was administered via a circle breathing system and anaesthesia maintained using an infusion pump with a constant rate infusion of alfaxalone (initially  $0.1\text{ mg kg}^{-1}\text{ minute}^{-1}$ ) during radiography, reducing to (initially)  $0.09\text{ mg kg}^{-1}\text{ minute}^{-1}$  for NWR testing. Adjustments were made to infusion rates to produce a clinically determined minimal anaesthetic state required for the procedure. It was important that dogs did not exhibit voluntary movement during the procedure, but a slight palpebral reflex was maintained. During anaesthesia dogs were constantly monitored by a trained research technician and a multiparameter monitor (Datex-Ohmeda S5; GE Medical Systems Ltd, UK) was used to measure HR,  $f_r$ , SAP, MAP,

DAP, end-tidal carbon dioxide (PE'CO<sub>2</sub>), and haemoglobin oxygen saturation (SpO<sub>2</sub>).

Hypoventilation (PE'CO<sub>2</sub> > 8 kPa recorded for at least 2 measurements) was managed by manually ventilating the lungs until PE'CO<sub>2</sub> < 6.7 kPa for 60 seconds and decreasing the rate of alfaxalone infusion if possible. Hypotension was defined as a MAP < 60 mmHg and was managed with a bolus of Hartmann's solution 5 mL kg<sup>-1</sup> intravenously.

Values for the monitored parameters were recorded at 5 minute intervals throughout the duration of anaesthesia. Blood glucose was measured hourly during anaesthesia using a hand-held blood glucose monitor calibrated for dogs (Alphatrak 2; Abbott Diabetes Care Inc., CA, USA) starting immediately after induction of anaesthesia. Blood for glucose measurement was collected from the left or right ear pinnae using a needle prick. Rectal temperature was monitored every 30 minutes and supported with insulated electric blankets. Hartmann's solution (Aquapharm no. 11; Animalcare, UK) was infused at a rate of 5 mL kg<sup>-1</sup> hour<sup>-1</sup> throughout anaesthesia.

#### Radiography and Nociceptive Withdrawal Reflex (NWR) testing

Radiography of the hindlimbs was performed over approximately 60 minutes. Dogs were positioned in left lateral recumbency for NWR testing and the protocol was performed as previously described (Hunt et al. 2016). Following NWR testing, the alfaxalone infusion was discontinued and the dogs constantly monitored. Recovery quality was scored using a simple descriptive scale (SDS) from 0 (poor) to 3 (excellent) (Hunt et al. 2014).

#### Statistical methods

Descriptive statistics were used to define the population studied. Raw data for physiological variables were plotted, and there appeared to be a change in recorded values at 60 minutes following induction, with a decrease in HR and increase in PE'CO<sub>2</sub> noted. Summary measures

(mean values) were thus calculated for physiological parameters for each dog from 0-60 minutes of anaesthesia, and from 60 minutes until discontinuation of anaesthesia, to produce three separate time periods for comparison; preanaesthesia (baseline), 0-60 minutes of anaesthesia, and from 60 minutes until discontinuation of anaesthesia. Physiological data were assessed using the Friedman test for repeated measures, followed by Dunn's multiple comparison post-hoc testing, to evaluate differences between time periods. Comparisons between paired data collected during two time periods only (e.g. PE'CO<sub>2</sub>, SpO<sub>2</sub>) were performed using Wilcoxon's signed rank test. Descriptive and physiological parameter data are presented as mean (95% CI), whilst SDS data are presented as median (25-75<sup>th</sup> percentile)

## **Results**

### Demographics

A total of 112 dogs completed anaesthesia out of 114 dogs eligible for the study.

Mean age was 9.2 (95% CI 8.8 - 9.7) years and weight was 26.7 (95% CI 24.9 - 28.4) kg.

Body condition score was a median of 5 (4-6). In total 10 male, 7 female, 43 male neutered, and 52 female neutered dogs completed anaesthesia. Mean induction dose of alfaxalone was 1.4 (95% CI 1.3 - 1.5) mg kg<sup>-1</sup>.

### Post-induction apnoea

Dogs which did not initiate spontaneous respiration within 60 seconds of induction of anaesthesia were administered manual ventilation at a rate of 4 breaths minute<sup>-1</sup>, until spontaneous ventilation resumed. Thirteen (11.6%) dogs required ventilation, for 3.5 (95% CI 3.3-3.6) minutes.



Duration of anaesthesia was 165 (150-195) minutes. The alfaxalone infusion rate during the first 60 minutes of anaesthesia was 0.1 (95% CI 0.09 - 0.1) mg kg<sup>-1</sup> minute<sup>-1</sup>; from 60 minutes until discontinuation of anaesthesia, the infusion rate was 0.1 (95% CI 0.08 - 0.09) mg kg<sup>-1</sup> minute<sup>-1</sup>.

#### Heart rate

Heart rate (Table 1) was significantly elevated [114 (95% CI 111 – 118) beats minute<sup>-1</sup>;  $p < 0.001$ ] during 0-60 minutes of anaesthesia compared with both baseline [98 (95% CI 94 – 103 beats minute<sup>-1</sup>)] and the second hour of anaesthesia [98 (95% CI 94 – 101 beats minute<sup>-1</sup>)].

#### Arterial blood pressure

Systolic, mean, and diastolic arterial blood pressure (Table 1) were significantly decreased during both time periods of alfaxalone anaesthesia, compared with baseline values, however, blood pressure was within physiological limits in the majority of animals. In 23 dogs (21%) a hypotension was recorded on at least 2 occasions during the first 60 minutes of anaesthesia, but only 9 dogs (8%) exhibited hypotension during anaesthesia from 60 minutes to discontinuation.

#### Respiratory rate

Frequency of respiration was significantly decreased during both time periods of alfaxalone anaesthesia; 11 (95% CI 10 - 12) breaths minute<sup>-1</sup> and 12 (95% CI 11 - 14) breaths minute<sup>-1</sup>, compared with preanaesthetic values of 22 (95% CI 21 - 23) breaths minute<sup>-1</sup> (Table 1).

#### End-tidal carbon dioxide and haemoglobin oxygen saturation

During the first 60 minutes of anaesthesia, the PE'CO<sub>2</sub> was 6 (95% CI 5.7 – 6.2) kPa, and significantly increased during the second hour of anaesthesia to 6.4 (95% CI 6.1 – 6.7) kPa;  $p < 0.001$ . Hypoventilation was identified in 18 dogs (16%).

### Glucose

Serum glucose concentration immediately following anaesthetic induction was 6.7 (95% CI 6.5-6.9) mmol L<sup>-1</sup> which was significantly higher than at initial examination [5.8 (95% CI 5.6-6.0) mmol L<sup>-1</sup>;  $p < 0.001$ ], but during anaesthesia serum blood glucose remained stable, compared with values immediately following induction { 1 hour [6.9 (95% CI 6.6 – 7.1) mmol L<sup>-1</sup>]; 2 hours [6.9 (95% CI 6.6 – 7.1) mmol L<sup>-1</sup>];  $p = 0.37$ ).

### Recovery

Median time between discontinuation of alfaxalone infusion and extubation, following the return of pharyngeal reflexes, was 17 (7 – 35) minutes. Time to assuming sternal recumbency was 75 (58 – 110) minutes, and time to standing was 109 (88 – 140) minutes. Recovery quality was good, with a SDS of 2 (2 – 3).

### Discussion

This prospective study demonstrates that an alfaxalone CRI provided effective anaesthetic maintenance to enable positioning for radiography, and for completion of a neurophysiological study in this population of client owned dogs, accompanied by similar physiological changes to those expected from previous research studies (Ambros et al. 2008; Suarez et al. 2012; Herbert et al. 2013).

Arterial blood pressure was decreased, compared with sedated, baseline values, whilst HR was increased during the first hour of anaesthesia. Other research has reported increases in

HR following induction of anaesthesia with alfaxalone, and it has been suggested that alfaxalone may better preserve baroreceptor function compared with propofol (Amengual et al. 2012).

Serum glucose concentration measured from ear capillary blood was found to be higher immediately following induction of anaesthesia, compared with a baseline measurement from a jugular vein blood sample one week prior to anaesthesia. It is most likely that anxiety, administration of premedicants and cephalic venous catheterisation prompted sympathetically mediated increases in glucagon and adrenaline, and decreases in insulin secretion; i.e. the increase in blood glucose concentration was likely promoted by a stress response to anaesthesia, including the administration of alfaxalone. The site of blood sampling may be expected to affect whole blood glucose concentrations, with arterial and capillary samples producing higher estimations compared to venous blood (Cengiz & Tamborlane 2009), and this may also have contributed to the apparent significant increase in glucose concentration between the screening appointment and anaesthesia. Despite changes from the screening appointment, glucose concentrations were stable throughout the period of anaesthesia, suggesting that HPCD neither significantly binds glucose, nor undergoes hydrolysis during CRI over a duration of 3 hours in the dog.

In conclusion, alfaxalone CRI at a rate of 0.09-0.1 mg kg<sup>-1</sup> minute<sup>-1</sup> produced stable anaesthesia for radiography and neurophysiological experiments in middle aged to older dogs with minimal clinically significant haemodynamic changes but was accompanied by respiratory depression. Endotracheal intubation and a means to deliver mechanical ventilation are essential for the safe use of the agent.

## Funding

This study was supported by the BBSRC grant number BB/L00240X/1. The alfaxalone was supplied by Jurox (UK) Limited. JRH, HJ, and MG positions were funded by the BBSRC grant number BB/L00240X/1. The authors declare no other conflict of interest.

## References

- Ambros B, Duke-Novakovski T, Pasloske, KS (2008) Comparison of the anesthetic efficacy and cardiopulmonary effects of continuous rate infusions of alfaxalone-2-hydroxypropyl-beta-cyclodextrin and propofol in dogs. *Am J Vet Res* 69, 1391–1398.
- Amengual M, Flaherty D, Auckburally et al. (2012) An evaluation of anaesthetic induction in healthy dogs using rapid intravenous injection of propofol or alfaxalone. *Vet Anaesth Analg* 40, 115–123.
- Cengiz E, Tamborlane, WV (2009) A tale of two compartments: interstitial versus blood glucose monitoring. *Diabetes Technol Ther* 11, S11–S16.
- Herbert GL, Bowlt KL, Ford-Fennah V et al. (2013) Alfaxalone for total intravenous anaesthesia in dogs undergoing ovariohysterectomy: a comparison of premedication with acepromazine or dexmedetomidine. *Vet Anaesth Analg* 40, 124–133.
- Hunt J, Murrell J, Knazovicky D (2016) Alfaxalone Anaesthesia Facilitates Electrophysiological Recordings of Nociceptive Withdrawal Reflexes in Dogs (*Canis familiaris*). *PLoS One* 11, p.e0158990.

Hunt JR, Slingsby LS, Murrell JC (2014). The effects of an intravenous bolus of dexmedetomidine following extubation in a mixed population of dogs undergoing general anaesthesia and surgery. *Vet J* 200, 133–139.

Irie T, Uekama K (1997) Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. *J Pharm Sci* 86, 147–162.

Muñoz KA, Robertson SA, Wilson DV (2017) Alfaxalone alone or combined with midazolam or ketamine in dogs: intubation dose and select physiologic effects. *Vet Anaesth Analg* 44, 766-774.

Suarez MA, Dziki BT, Stegmann FG et al. (2012) Comparison of alfaxalone and propofol administered as total intravenous anaesthesia for ovariohysterectomy in dogs. *Vet Anaesth Analg* 39, 236–244.

**Table 1** Physiological measures compared over time in dogs premedicated with acepromazine and induced with intravenous alfaxalone and maintained with an intravenous alfaxalone continuous rate infusion. Data are reported as mean (95% CI).

Variable	Preanaesthetic	Anaesthesia 0-60 minutes	Anaesthesia 60 minutes - discontinuation	p
HR (beats minute <sup>-1</sup> )	98 (95% CI 94-103) <sup>a</sup>	114 (95% CI 111-118) <sup>b</sup>	98 (95% CI 94 – 101) <sup>a</sup>	<0.001***
$f_R$ (breaths minute <sup>-1</sup> )	22 (95% CI 21-23) <sup>a</sup>	11 (95% CI 10-12) <sup>b</sup>	12 (95% CI 11-14) <sup>b</sup>	<0.001***
SAP (mmHg)	128 (95% CI 122-133) <sup>a</sup>	109 (95% CI 106-111) <sup>b</sup>	112 (95% CI 109-114) <sup>b</sup>	<0.001***
MAP (mmHg)	100 (95% CI 95 – 105) <sup>a</sup>	83 (95% CI 81-85) <sup>b</sup>	82 (95% CI 80-84) <sup>b</sup>	<0.001***
DAP (mmHg)	81 (95% CI 76-86) <sup>a</sup>	66 (95% CI 63-68) <sup>b</sup>	63 (95% CI 61-66) <sup>b</sup>	<0.001***
PE'CO <sub>2</sub> (kPa)	-	6 (95% CI 5.7-6.2)	6.4 (95% CI 6.1-6.7)	<0.001***
S <sub>p</sub> O <sub>2</sub> (%)	-	98 (95% CI 97-98)	99 (95% CI 99-99)	<0.001***
Rectal Temperature (°C)	38.3 (95% CI 38.1 – 38.5) <sup>a</sup>	37.6 (95% CI 37.5 – 37.7) <sup>b</sup>	37.5 (95% CI 37.4 – 37.7) <sup>b</sup>	<0.001***

Superscript letters indicate groupings within the data, shared superscripts indicate no significant difference between groups on post-hoc testing, differing superscripts indicate a difference with a  $p$  value of less than 0.05 on post-hoc testing. \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$