RESEARCH PAPER

Effects of buprenorphine, butorphanol or tramadol premedication on anaesthetic induction with alfaxalone in common marmosets (*Callithrix jacchus*)

Jaco Bakker[a], Sandra Roubos[b], Edmond J Remarque[c], Saskia S Arndt[b], Peter W Kronen[d,1] & Jan AM Langermans[a,1]

[a]Animal Science Department, Biomedical Primate Research Centre, Rijswijk, The Netherlands
[b]Department of Animals in Science & Society, Division of Animal Welfare & Laboratory Animal Science, Faculty of Veterinary Medicine Utrecht University, Utrecht, The Netherlands
[c]Department of Parasitology, Biomedical Primate Research Centre, Rijswijk, The Netherlands
[d]Veterinary Anaesthesia Services – International, Winterthur, Switzerland

Correspondence. Jaco Bakker, Animal Science Department, Biomedical Primate Research Centre, 2288GJ Rijswijk, The Netherlands. E-mail: bakker@bprc.nl
[1] These authors share senior authorship.

Abstract

Objective To investigate the clinical and physiological effects of intravenous (IV) alfaxalone alone or in combination with buprenorphine, butorphanol or tramadol premedication in marmosets.

Study design Prospective, randomized, blinded, crossover design.

Animals Nine healthy marmosets (391 ± 48 g, 3.7 ± 2.2 years old).

Methods Meloxicam 0.20 mg kg^{-1} subcutaneously, atropine 0.05 mg kg^{-1} intramuscularly (IM) and either buprenorphine 20 μg kg^{-1} IM (BUP-A), butorphanol 0.2 mg kg^{-1} IM (BUT-A), tramadol 1.5 mg kg^{-1} IM (TRA-A) or no additional drug (control) were administered to all marmosets as premedication. After 1 hour, anaesthesia was induced with 16 mg kg^{-1} alfaxalone IV. All animals received all protocols. The order of protocol allocation was randomized with a minimum 28 day wash-out period. During anaesthesia, respiratory and pulse rates, rectal temperature, haemoglobin oxygen saturation, arterial blood pressure, palpebral and pedal withdrawal reflexes and degree of muscle relaxation were assessed and recorded every 5 minutes. Quality of induction and recovery were assessed. Duration of induction, immobilization and recovery were recorded. Blood samples were analysed for aspartate aminotransferase, creatine kinase and lactate dehydrogenase concentrations. The protocols were compared using paired *t* tests, Wilcoxon's signed-rank test with Bonferroni's corrections and linear mixed effect models where appropriate.

Results Out of nine animals, apnoea was noted in eight animals administered protocol BUP-A and two animals administered protocol BUT-A. With TRA-A and control protocols, apnoea was not observed. No other significant differences in any of the parameters were found; however, low arterial blood pressures and hypoxia occurred in TRA-A.

Conclusions and clinical relevance Our study employing different premedications suggests that the previously published dose of 16 mg kg^{-1} alfaxalone is too high when used with premedication because we found a high incidence of complications including apnoea (BUP-A), hypotension and hypoxaemia (TRA-A). Appropriate monitoring and countermeasures are recommended.

Keywords alfaxalone, anaesthesia, marmosets, premedication.

Introduction

Marmosets are regularly used as models in neuroanatomical and neurophysiological studies for biomedical research. Often this requires the implantation of devices, such as for telemetry (Pearce et al.

1998; Crofts et al. 2001; Philippens & Vanwersch 2010). The implantation of such devices is an invasive surgery. The anaesthetics used to perform such a surgery should have a wide safety margin, lack local irritant properties, allow for a rapid and complete return of consciousness and appetite, provide adequate muscle relaxation and should not accumulate in the body. Alfaxalone fulfils these criteria for marmosets (Bakker et al. 2013) and is widely used for the induction and maintenance of general anaesthesia in several species (Grint et al. 2008; Muir et al. 2008; Whittem et al. 2008).

However, alfaxalone produces sedative and anaesthetic effects with no antinociception (Nadeson & Goodchild 2000; Kalchofner Guerrero et al. 2014). Therefore, pre- and postoperative analgesia must be provided when major surgery is performed. When selecting the appropriate analgesic, potential side effects must be considered. It is important to establish whether clinically significant interactions occur between the analgesic agent used and the anaesthetic regime. Of particular concern are respiratory depression and prolonged recovery times (Dahan 2006).

Reports of non-human primate experiments rarely include details regarding possible adverse events related to interactions of the administered anaesthetics and analgesics (Liguori et al. 1996), and to the authors' knowledge, no such reports with marmosets are available. The objective of this study was to evaluate clinical and cardiorespiratory effects of intramuscular (IM) buprenorphine, butorphanol or tramadol administration prior to anaesthetic induction with intravenous (IV) alfaxalone. In this study, the drug dosages and combinations were chosen according to the institute's practices and published data (Prestes et al. 2014; Grimm et al. 2015; Kelly et al. 2015).

Material and methods

Animals, housing and care

Ethics approval was obtained from the Animal Experiments Committee (DEC) of the Biomedical Primate Research Centre (BPRC, Rijswijk, The Netherlands) prior to the commencement of the study (DEC-BPRC number: #759). The procedures performed in this study were in accordance with the Dutch laws on animal experimentation, with the regulations for animal handling as described in the EU Directive 63/2010 and with the Weatherall report (2006). BPRC is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

The sample size calculation was based on recovery time from anaesthesia (primary outcome measure). Considering an alpha of 0.05, beta of 0.2 (80% power) and the minimal detectable difference between paired observations (Student's t test) to be 1.41 × standard deviation (SD), a group size of nine animals was suggested. The minimal detectable difference was calculated from a previous study using alfaxalone in marmosets (Bakker et al. 2013). In this earlier study, the mean recovery time difference between the groups varied between 1.3 and 5.8 hours with SD values between 0.5 and 1.3 hours. It was anticipated that with nine animals (Microsoft R open 3.3.2; R Foundation for Statistical Computing, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org), a significant difference in recovery time could be found between the different protocols.

Nine healthy adult common marmosets (*Callithrix jacchus*), four males and five females, were included in the study. A complete physical, haematological, and biochemical examination was performed on all animals prior to the study. Animals originated from and were housed at BPRC. The animals were familiar with the procedures and personnel involved. They remained under veterinary supervision during the entire study period. Animals were housed as same-sex pairs in cages (150 × 75 × 185 cm) enriched with branches and toys. Room temperature was controlled between 23.8–26.5 °C, with a 12 hour light/dark cycle per day (artificial lighting). Animals were fed commercial monkey pellets (Ssniff; Soest, Germany) *ad libitum* with Arabic gum supplementation and limited amounts of fresh fruit. Additional food and non-food enrichment was provided regularly. Drinking water was provided *ad libitum* in water bottles. Water intake was never restricted, but food was withheld for 16 hours prior to anaesthesia. After completion of the study, the animals were returned to the marmoset research colony of BPRC.

Study design

This study was conducted from January to October 2015. Four protocols were compared in a crossover design with a minimum 28 day wash-out period. Each animal was administered each protocol once, and the protocol sequences were randomly assigned to each animal using R-software.

The animals were weighed prior to each anaesthesia. On the two days following each anaesthesia,

body weight was measured daily to determine a possible effect of anaesthesia on body weight. Animals were taken out of their cage by means of a Perspex cylinder. The cylinder with the animal in it was placed on a scale as a noninvasive method of assessing the body weight.

Animals were administered 0.20 mg kg^{-1} meloxicam subcutaneously (SC, Metacam 2 mg mL^{-1}; Boehringer Ingelheim B.V., The Netherlands) and 0.05 mg kg^{-1} atropine intramuscularly (IM, Atropine sulphate PCh 1 mg mL^{-1}; Pharmachemie B.V., The Netherlands). According to the protocol allocation for that day, they were also administered one of the following analgesic premedications IM: 20 µg kg^{-1} buprenorphine (Buprecare 0.3 mg mL^{-1}; AST Farma B.V., The Netherlands; protocol BUP-A); 0.20 mg kg^{-1} butorphanol (Torbugesic Vet 10 mg mL^{-1}; Zoetis B.V., The Netherlands; protocol BUT-A); 1.5 mg kg^{-1} tramadol (Tramal 100; Grünenthal GmbH, Germany; protocol TRA-A); or no injection (control protocol).

For administration of the premedication, one person manually restrained the animal while a second person administered the drug volume IM and SC into the left or right quadriceps femoris and into the subcutis of the abdomen, respectively, using 26 gauge needles. Care was taken that the drugs were not injected intravascularly. Once premedicated, the animals were released into their home cage with their social partner and left undisturbed for 60 minutes. During this time, they were monitored until they were re-restrained to induce anaesthesia. The observer, performing the quality scoring of the induction and recovery period and the anaesthetic monitoring, was unaware of the protocol and did not observe drug administrations.

An hour after the premedication, sampling of approximately 200 µL blood was performed via direct venipuncture of the left or right cephalic vein using a 26 gauge needle. Samples were collected with one person restraining the animal while a second person performed the blood sampling. The samples were processed immediately with a Cobas Integra 400 plus analyser (F. Hoffmann-La Roche Ltd., Switzerland). Levels of aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH) were determined in serum. Samples were taken from sedated animals just before alfaxalone administration and 24 and 48 hours after alfaxalone administration. Control samples were collected 28, 27 and 26 days prior to the first anaesthetic and on day 28, 29 and 30 after the last anaesthetic.

Anaesthesia was induced by administration of 16 mg kg^{-1} alfaxalone (Alfaxan; Vetoquinol B.V., The Netherlands) IV over 90 seconds. To allow for comparisons among the premedication protocols, the full dose was administered in all cases. No other procedures than what is described were performed on the animals.

A heating pad (ThermoluxWärmematten; Witte + Sutor GmbH, Germany) was placed under the animal during the immobilization period. Eye drops (Viadrops; Ceva Santé Animale B.V., The Netherlands) were applied onto the corneas at the start of the immobilization period.

Tracheal intubation was not performed, and the animals were allowed to breathe spontaneously in room air throughout the protocols.

Anaesthetic monitoring

For each protocol, induction (Induc-T), immobilization (Immob-T) and recovery times (Recov-T) were recorded. Induc-T was defined as the time between start of the injection of alfaxalone and loss of righting reflex. Immob-T was defined as the time from the loss of righting reflex to the animal's first attempt to lift its head. Recov-T was defined as the time from the animal's first attempt to lift its head until the moment that the animal could walk and climb confidently in the restricted confines of its cage and could be reunited safely with its companion. Total procedure time (TP-T) was calculated as the sum of Induc-T, Immob-T and Recov-T.

The quality of induction was assessed (Appendix A) using a previously published ordinal scale (Bakker et al. 2013). Time point T0 was the moment of alfaxalone injection. During the immobilization period, the following variables were measured and recorded every 5 minutes (T5, T10, T15 and so on) by an observer who was unaware of the choice of protocol. The pulse rate (PR), systolic (SAP), diastolic (DAP) and mean (MAP) arterial pressures were recorded using a noninvasive, oscillometric device (vetHDO monitor with MDSoftware; S+B medVet GmbH, Germany) with a cuff (Criticon Soft-cuff size 1; GE Healthcare, The Netherlands) placed on the left upper arm of the animal (*regio brachii*). Percentage of peripheral haemoglobin oxygen saturation (SpO_2) was measured using the earlobe clip of a veterinary pulse oximeter (Ohmeda biox 3740; BOC Health Care, Inc., KY, USA) positioned on the right hand. Respiratory frequency (f_R) was determined by observing thoracic excursions over a 30 second

period. Rectal temperature (T) was measured using a digital thermometer (Microlife Vettemp; Microlife AG, Switzerland) with a measurement range of 32.0–42.9 °C.

Apnoea was defined as no respiratory movements for 30 seconds. If apnoea occurred, 100% oxygen was provided with a flow of 0.5 L minute^{-1} via a face mask until the end of the immobilization period. Simultaneously, gentle external intermittent manual compression of the thorax was applied (interval: six consecutive compressions of 2 seconds every 30 seconds during the apnoea) until the animal started to breathe spontaneously again.

At the end of the immobilization period, the animal recovered in its cage with the warming blanket placed under the animal to preserve body temperature. The quality of recovery was assessed using a previously published ordinal scale (Bakker et al. 2013; Appendix A). In the subsequent months, behaviour, social interaction and appetite were scored to monitor for possible long-term effects.

Assessment of anaesthetic depth

Clinical criteria used to assess anaesthetic depth consisted of palpebral reflex, muscle tension and withdrawal reflex. Recordings were made every 5 minutes after the start of the immobilization period until the end of the immobilization period. The presence or absence of the palpebral reflex was tested by lightly touching the medial canthus of an eye with a dry cotton swab without touching the cornea. For animals experiencing apnoea, palpebral reflexes were not assessed because of the presence of the face mask. Muscle tone was scored using an ordinal scale by judging the resistance of the left leg when pulled. The pedal withdrawal reflex was determined by applying a haemostat closed to the first ratchet for second on the third phalanx of the left leg (Appendix B). Testing for analgesic efficiency was not a part of this study.

Statistical analysis

All statistical analyses were performed using the R language and environment for statistical computing Microsoft R open 3.3.2. To determine statistical significance between protocols in Induc-T, Immob-T and Recov-T, paired *t* tests were performed. Data on measured physiological parameters (PR, SAP, DAP, MAP, f_R, T and SpO_2) were tested for significant differences using the Wilcoxon signed-rank test. To adjust for multiple tests, a Bonferroni correction was applied. Clinical chemistry values (AST, LDH and CK) and body weight were analysed with linear mixed effect models. A value of $p < 0.05$ was considered significant.

Results

The body weights of the animals were 391 ± 48 g and their age was 3.7 ± 2.2 years. All animals were healthy on physical examination, and all blood values were within the normal range before entering the study.

Two animals in the control protocol, one in TRA-A and one in BUT-A recovered at 40–45 minutes; therefore, detailed comparison of all protocols was performed for the first 45 minutes only (Table 1). No animals or data were excluded.

None of the animals showed signs of pain during or after injection of the premedication with any protocol. After administration of butorphanol, all the nine animals showed depression of awareness to the environment and reduction of their responsiveness to external stimulation. After tramadol administration, four out of nine animals showed exaggerated tongue flicking, foaming at the mouth and mouth wiping in the first 10 minutes after administration only.

The quality of induction was rated good in all protocols (Appendix A). After alfaxalone was administered, apnoea occurred for 27 ± 18 minutes in eight out of nine animals administered BUP-A (range, 8 – 63 minutes) and two out of nine animals administered BUT-A [both 14 minutes]. The control protocol and TRA-A did not result in apnoea. All incidents of apnoea occurred within 2 and 5 minutes after initiation of the alfaxalone administration (immobilization phase) in the BUP-A and BUT-A protocols, respectively. A sharp drop in SpO_2 was observed in every case immediately after apnoea. Treatment with supplemental oxygen and manual compression of the thorax resulted in a significant re-increase of SpO_2 (Table 1). All animals were immobilized before the full alfaxalone dose was administered.

Clinical data of the first 45 minutes of immobilization are shown in Table 1. The SpO_2 was initially depressed in animals showing apnoea but increased to 90–100% when oxygen was supplied in combination with manual compression of the thorax. Apart from f_R and SpO_2 in the apnoeic animals, no significant differences in any of the recorded parameters were detected between protocols at any time point during the immobilization period.

Table 1 Cardiorespiratory parameters measured during the first 45 minutes of the immobilization phase of each protocol. The number of observations is nine unless specified, $^{*}n = 8$, $^{**}n = 7$, and $^{***}n = 6$. Four different protocols were administered to nine marmosets in a crossover design: 0.20 mg kg^{-1} meloxicam subcutaneously and 0.05 mg kg^{-1} atropine intramuscularly (IM) followed by premedication with buprenorphine (20 μg kg^{-1}; protocol BUP-A); butorphanol (0.2 mg kg^{-1}; protocol BUT-A); tramadol (1.5 mg kg^{-1}; protocol TRA-A) or no injection (control group) IM in a crossover design. After 1 hour, anaesthesia was induced by intravenous administration of 16 mg kg^{-1} alfaxalone over 90 seconds

	Protocol	Time points								
		T5	T10	T15	T20	T25	T30	T35	T40	T45
f_R (breaths minute^{-1})	Control	34 ± 13	36 ± 13	38 ± 10	40 ± 13	40 ± 13	40 ± 12	42 ± 14	42 ± 14	37 ± 9**
	BUP-A	3 ± 8**	5 ± 9*	7 ± 13*	11 ± 13*	12 ± 13	15 ± 12	15 ± 11	16 ± 14	17 ± 13
	BUT-A	13 ± 9	20 ± 18	24 ± 21	29 ± 18	31 ± 17	32 ± 16	33 ± 16	33 ± 16	30 ± 15*
	TRA-A	29 ± 15	32 ± 15	35 ± 13	36 ± 13	36 ± 11	36 ± 12	36 ± 12	39 ± 11	39 ± 10*
SpO_2 (%)	Control	86 ± 7	90 ± 4	92 ± 3	94 ± 2	94 ± 2	95 ± 2	94 ± 3	94 ± 4	96 ± 2**
	BUP-A	73 ± 33**	78 ± 22*	95 ± 9	95 ± 8	95 ± 7	95 ± 6	92 ± 9	90 ± 10	89 ± 12
	BUT-A	53 ± 21	67 ± 21	79 ± 13	85 ± 9	88 ± 7	90 ± 5	91 ± 5	93 ± 4	94 ± 4*
	TRA-A	78 ± 10	84 ± 7	89 ± 05	92 ± 4	94 ± 3	94 ± 3	95 ± 3	95 ± 3	94 ± 6*
T (°C)	Control	38.4 ± 0.4	38.0 ± 0.4	37.8 ± 0.4	37.5 ± 0.4	37.2 ± 0.5	37.1 ± 0.4	36.9 ± 0.4	36.8 ± 0.5	36.5 ± 0.4**
	BUP-A	37.8 ± 0.9**	37.3 ± 0.7*	37.1 ± 0.6*	36.8 ± 0.5*	36.6 ± 0.4	36.4 ± 0.4	36.2 ± 0.5	36.1 ± 0.5	36.0 ± 0.5
	BUT-A	37.2 ± 0.9	36.9 ± 0.8	36.6 ± 0.8	36.5 ± 0.7	36.4 ± 0.7	36.3 ± 0.7	36.2 ± 0.6	36.2 ± 0.6	36.2 ± 0.7*
	TRA-A	38.1 ± 0.4	37.6 ± 0.4	37.2 ± 0.5	36.8 ± 0.5	36.5 ± 0.5	36.2 ± 0.5	35.9 ± 0.6	35.7 ± 0.6	35.6 ± 0.7*
SAP (mmHg)	Control	78 ± 10	76 ± 7	75 ± 6	78 ± 7	79 ± 7	83 ± 10	87 ± 15	89 ± 15**	88 ± 14***
	BUP-A	123 ± 41	89 ± 16*	87 ± 10*	87 ± 9	89 ± 9	92 ± 8	94 ± 11	106 ± 26	101 ± 9
	BUT-A	84 ± 33*	75 ± 7	78 ± 9	80 ± 9	83 ± 7	87 ± 9	89 ± 11	94 ± 12	93 ± 11*
	TRA-A	79 ± 14	79 ± 13	81 ± 14	80 ± 11	83 ± 12	81 ± 9	88 ± 11	96 ± 16	98 ± 10**
DAP (mmHg)	Control	44 ± 7	46 ± 3	46 ± 6	46 ± 5	44 ± 7	45 ± 7	50 ± 13	50 ± 9**	45 ± 7***
	BUP-A	64 ± 26	52 ± 10*	50 ± 4*	51 ± 5	50 ± 7	50 ± 5	50 ± 6	52 ± 6	53 ± 5
	BUT-A	48 ± 16*	45 ± 6	43 ± 6	45 ± 6	45 ± 6	47 ± 6	49 ± 6	52 ± 8	51 ± 9*
	TRA-A	41 ± 6	44 ± 10	45 ± 10	46 ± 7	45 ± 9	43 ± 4	47 ± 6	51 ± 9	50 ± 14**
MAP (mmHg)	Control	57 ± 6	58 ± 4	57 ± 6	58 ± 5	57 ± 6	59 ± 7	64 ± 14	64 ± 11**	61 ± 9***
	BUP-A	85 ± 30	66 ± 11*	64 ± 5*	64 ± 5	65 ± 7	65 ± 5	66 ± 6	71 ± 12	70 ± 6
	BUT-A	61 ± 21*	56 ± 6	56 ± 7	58 ± 6	59 ± 5	62 ± 6	64 ± 7	68 ± 9	66 ± 9*
	TRA-A	55 ± 8	57 ± 10	58 ± 10	59 ± 8	58 ± 8	57 ± 4	62 ± 7	67 ± 11	67 ± 11**
PR (beats minute^{-1})	Control	310 ± 36	295 ± 33	279 ± 30	260 ± 26	252 ± 26	250 ± 29	232 ± 38	249 ± 40**	231 ± 35***
	BUP-A	318 ± 51	309 ± 57	327 ± 39	327 ± 41	323 ± 42	311 ± 43	302 ± 53	279 ± 67	291 ± 59
	BUT-A	306 ± 73*	311 ± 18*	327 ± 32*	331 ± 37	332 ± 39	331 ± 41	334 ± 40	339 ± 39	332 ± 37*
	TRA-A	331 ± 32	307 ± 26	292 ± 24	279 ± 26	268 ± 26	259 ± 27	252 ± 29	257 ± 42	269 ± 45**

DAP, diastolic blood pressure; f_R, respiratory rate; MAP, mean blood pressure; PR, pulse rate; SAP, systolic blood pressure; SpO_2, haemoglobin oxygen saturation; T, rectal temperature. Each timepoint (T5–T45) represents data recording at respective 5 minute intervals.

The animals' body temperatures at the beginning of the immobilization period were between 36.0 °C and 39.4 °C. The temperature progressively tended to decrease during all protocols. At the end of the immobilization period, measured body temperatures were between 33.6 °C and 37.5 °C (Table 1). There was a direct relationship between duration of the immobilization and decrease in body temperature. The longer the immobilization period, lower the body temperature.

Induc-T, Immob-T, Recov-T and TP-T times are presented in Table 2. At Immob-T, most animals scored a zero for muscle tension, palpebral reflex and withdrawal reflex (96 out of 98 performed reflex tests; data not shown) at T5. No significant differences between the protocols were observed regarding muscle tension, palpebral and withdrawal reflexes. However, BUP-A and BUT-A premedication seemed to extend the duration of muscle relaxation compared to the TRA-A and control protocol. Four animals still had no pedal withdrawal reflex after 45 minutes of immobilization. All animals in the control protocol displayed muscle twitches near the end of the immobilization phase.

The results of the quality assessment of the recovery period (Table 3) showed no significant differences between the protocols. Quality of recovery did not differ between non-apnoeic animals and apnoeic animals and between animals having high SpO_2 values and low SpO_2 values. All animals, including the apnoeic animals and animals with low SpO_2, showed normal behaviour and appetite directly after end of the recovery period. No alterations in behaviour, social interaction and appetite were observed over the subsequent months.

CK, LDH and AST levels were not significantly affected following anaesthesia (p values between 0.2 and 0.9; Fig. 1a–c). However, day zero values of all anaesthetic protocols showed higher absolute CK values than samples taken 28 days before and 28 days after the protocols (data not shown).

No change in body weight, food or water intake following anaesthesia was observed. The body weights did not differ significantly from baseline values for any of the protocols under investigation.

Discussion

This study investigated some clinical and physiological effects of IV alfaxalone alone or in combination with buprenorphine, butorphanol or tramadol premedication in marmosets.

At the doses employed here, we found a high incidence of complications. This regards particularly the occurrence of apnoea (8/9 animals in BUP-A and 2/9 animals in BUT-A). Therefore, we advise caution when applying these protocols and cannot recommend the BUP-A protocol, as employed in our study. Animals need to be carefully observed for occurrence of apnoea and material for manual ventilation should be ready if needed. Apnoea did not occur in the control protocol or in a previously published study using alfaxalone (Bakker et al. 2013). The premedication doses employed in this study have not been previously published in marmosets but represent clinically used doses in many other species (following the instructions of the drug manufacturer) and are commonly used to provide varying degrees of analgesia (Flecknell 2009).

The induction times produced by the protocols in this study were very short (in all protocols well below

Table 2 Induction time (Induc-T), immobilization time (Immob-T), recovery time (Recov-T) and total procedure time (TP-T). Four different protocols were administered to nine marmosets in a crossover design: 0.20 mg kg^{-1} meloxicam subcutaneously and 0.05 mg kg^{-1} atropine intramuscularly (IM) followed by premedication with buprenorphine (20 µg kg^{-1}; protocol BUP-A); butorphanol (0.2 mg kg^{-1}; protocol BUT-A); tramadol (1.5 mg kg^{-1}; protocol TRA-A) or no injection (control group) IM in a crossover design. After 1 hour, anaesthesia was induced by intravenous administration of 16 mg kg^{-1} alfaxalone over 90 seconds

Protocol	Time (minutes)			
	Induc-T	Immob-T	Recov-T	TP-T
Control	0.4 ± 0.3	67.3 ± 26.5	43.8 ± 13.9	111.4 ± 36.1
BUP-A	0.2 ± 0.1	59.9 ± 11.8	36.8 ± 13.0	96.9 ± 19.2
BUT-A	0.1 ± 0.0	68.2 ± 22.5	65.2 ± 15.9	133.6 ± 34.1
TRA-A	0.1 ± 0.0	65.7 ± 15.8	32.7 ± 10.5	98.6 ± 16.0

Induc-T, time between start of the injection of alfaxalone and loss of righting reflex; Immob-T, time from the loss of righting reflex to the animal's first attempt to lift its head; Recov-T, time from the animal's first attempt to lift its head until the moment that the marmoset could walk and climb confidently in the restricted confines of its cage; TP-T was calculated as the sum of Induc-T, Immob-T, and Recov-T.

Table 3 Results of the quality assessment score of recovery period represented as number of marmosets with each protocol. The four different protocols were administered to nine marmosets in a crossover design: 0.20 mg kg^{-1} meloxicam subcutaneously and 0.05 mg kg^{-1} atropine intramuscularly (IM) followed by premedication with buprenorphine (20 µg kg^{-1}; protocol BUP-A); butorphanol (0.2 mg kg^{-1}; protocol BUT-A); tramadol (1.5 mg kg^{-1}; protocol TRA-A) or no injection (control group) IM in a crossover design. After 1 hour, anaesthesia was induced by intravenous administration of 16 mg kg^{-1} alfaxalone over 90 seconds

Protocol	Score 1 (good)	Score 2 (satisfactory)	Score 3 (unsatisfactory)
	Number of animals	Number of animals	Number of animals
Control	5	3	1
BUP-A	8	1	0
BUT-A	5	2	2
TRA-A	9	0	0

Score 1: no vocalisation, salivation and compulsive licking or sneezing, aware of environment.
Score 2: some vocalisation, salivation, compulsive licking, sneezing, some stereotypical behaviour but periods of awareness.
Score 3: excessive salivation, vomiting, compulsive licking, sneezing, profound stereotypical behaviour (the animals mostly moved in a circular pattern at the boundary of their enclosure while being unaware of its environment), unaware of environment.

30 seconds), as was the case in a previous study also using 16 mg kg^{-1} alfaxalone (Bakker et al. 2013), suggesting that this alfaxalone dose could be reduced. Both induction and recovery from anaesthesia, however, seemed smooth and complication-free making it useful for clinical and experimental procedures. One should keep in mind that alfaxalone is not described to have antinociceptive properties. Our study was not designed to test for antinociception; instead, we wanted to test the effects of combining drugs considered to be analgesics with alfaxalone such that quantitative antinociceptive testing could be studied subsequently. This remains an important future task. We hypothesize that the incidence of apnoea will decrease if a lower dose of alfaxalone is used after premedication with buprenorphine or butorphanol.

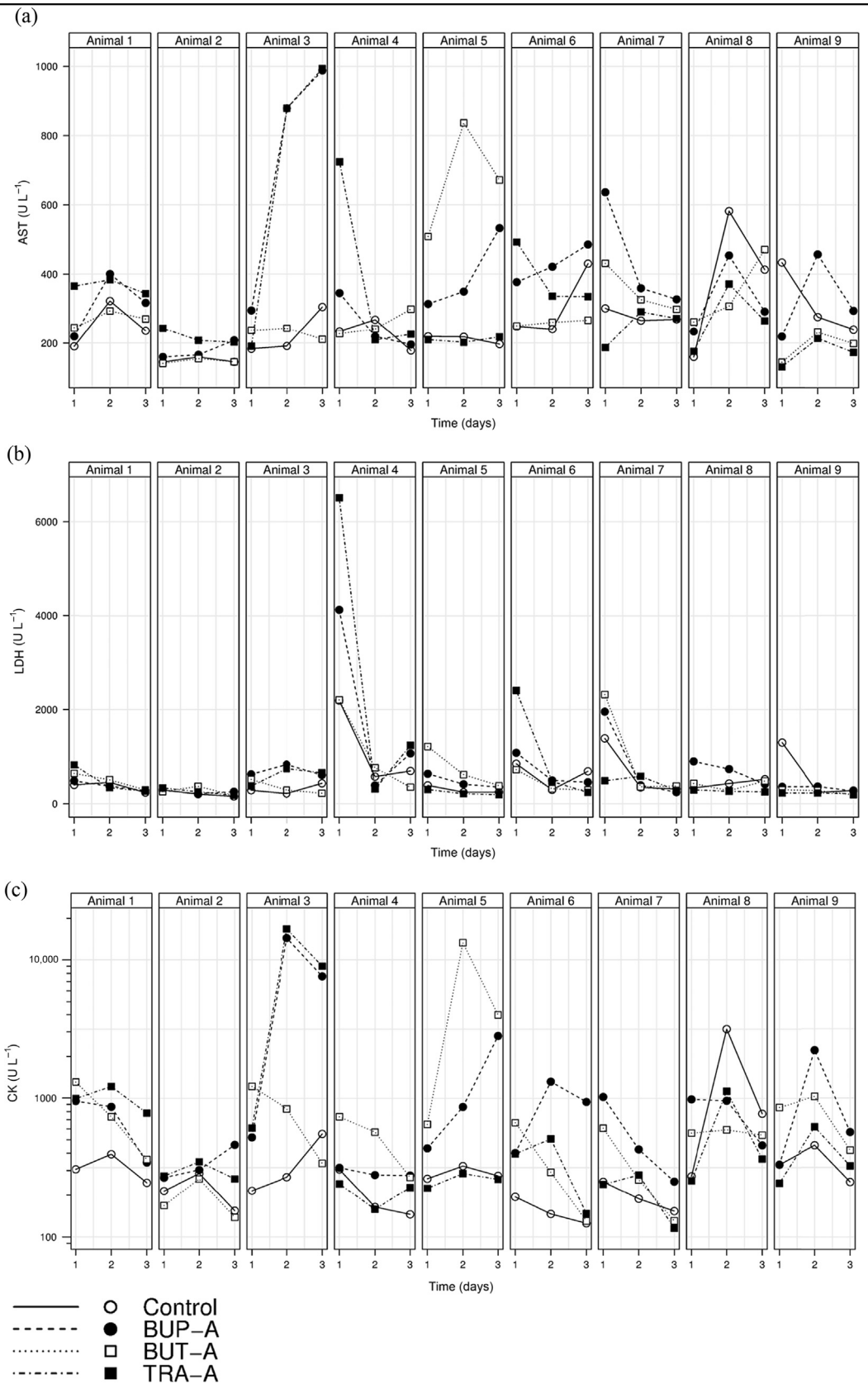
A similar high incidence of apnoea was described in rabbits: nine out of 10 rabbits experienced apnoea following IV administration of alfaxalone after premedication with morphine (Navarrete-Calvo et al. 2014). In dogs, apnoea was commonly observed following IV administration of alfaxalone after premedication with buprenorphine (Herbert et al. 2013). Thus, our data and earlier published data demonstrate that administration of IV alfaxalone after premedication with IM opioids may result in apnoea.

In dogs, among others, apnoea resulted from rapid IV administration of alfaxalone (Grint et al. 2008; Muir et al. 2009; Herbert et al. 2013; Lau et al. 2013; Giral et al. 2014; Navarrete-Calvo et al. 2014). In addition, dose-dependent respiratory depression after administration of alfaxalone has been observed in studies involving rats, cats, dogs and green iguanas (Muir et al. 2008; Muir et al. 2009; Bertelsen & Sauer 2011; Keates & Whittem 2012; Herbert et al. 2013; Lau et al. 2013).

We used an alfaxalone dose of 16 mg kg^{-1} as a dose of 12 mg kg^{-1} was suggested only to be somewhat useful for sedation to perform painless procedures requiring less than 15 minutes in marmosets (Bakker et al. 2013). The dose of alfaxalone (control protocol) used here was sufficient to produce a surgical plane of anaesthesia for 20–30 minutes.

One advantage of administrating premedication is dose reduction of the induction agent. In our study, it is likely that a lower dose of alfaxalone would have been sufficient to induce and maintain anaesthesia because all animals were immobilized before the full alfaxalone dose was administered. However, to allow comparison of the collected data afterwards, we administered the same dose of alfaxalone to all animals. A follow-up study investigating the degree of sedation after premedication and incidence of side effects while administering alfaxalone 'to effect' might resolve this question.

In animals that did become apnoeic, we immediately administered supplemental oxygen via face mask and compressed the thorax externally to achieve a degree of pulmonary ventilation. In fact, when doing so, SpO_2 measurements returned to 90–100%. We assumed potential hypoxia to be reversed to normoxia. No cyanosis of the visible mucous membranes was observed with any protocol at any time and not even when SpO_2 was low; however, visible cyanosis is not considered a reliable way of assessing the degree of arterial oxygenation.



As animals were not endotracheally intubated, end-tidal carbon dioxide measurement and proper artificial ventilation of the lungs were not possible. Also, arterial blood gas analysis was not available for this study and is technically difficult to perform in marmosets because of their small size. It would, however, have helped to assess the degree of hypoxia and respiratory acidosis.

The buprenorphine dose used in this study for marmosets was higher than that used in earlier reports (Hawk and Leary, 1999; Flecknell 2009). In the absence of clear dose recommendations for marmosets, we followed the dose advised in the product data sheet for cats and dogs. Moreover, it was not a goal of this study to investigate the analgesic effects of the used analgesics and analgesiometric studies are needed.

The blood pressure values obtained in this study were lower than previously published data for noninvasive blood pressure readings in marmosets (Schnell & Wood 1993; Mietsch & Einspanier 2015). We attribute this to the doses of alfaxalone and buprenorphine used which may have been too high and contributed to the cardiorespiratory side effects.

As this was the first study to assess cardiorespiratory parameters after alfaxalone administration with or without premedication, we believe, however, that the data from measurements of noninvasive blood pressure, f_R, SpO_2, T and PR marmosets anaesthetized with alfaxalone provide valuable information for future studies and clinical anaesthesia.

Although a heating pad was used, most animals developed a degree of hypothermia. Suppression of thermoregulatory defence mechanisms during general anaesthesia is dose dependent and mostly results in perioperative hypothermia (Sessler 1997; Lenhardt 2010). The use of more effective warming devices, such as forced air warming, might have prevented this fall of body temperature.

To determine possible local myotoxic effects of the injected formulations (premedication and anaesthetics), blood samples were analysed for CK. No significant differences in CK levels indicating muscle damage were observed between protocols. The slight increase in CK at day 0 of the protocols compared to the control values at day 0 of blood samples taken 28 days before and 28 days after the protocols (data not shown) remains unexplained but is considered to be clinically nonsignificant.

The palpebral reflex was used to test anaesthetic depth (loss of consciousness), the muscle tension is thought to test the degree of muscle relaxation and the pedal withdrawal reflex is a nociceptive withdrawal reflex and as such a test for analgesia. However, data of the observed muscle tension, palpebral reflex and pedal withdrawal reflex showed no significant differences between the protocols and are therefore hard to interpret. A follow-up study investigating the degree of sedation and level of analgesia are needed.

Conclusions

Based on the high incidence of complications, we cannot recommend the protocols employing buprenorphine, butorphanol or tramadol together with alfaxalone at the doses used if material for intubation and manual ventilation are not available. Future studies should investigate using a lower dose of alfaxalone in combination with these premedication protocols

Acknowledgements

The authors would like to thank Nathalie Wissink-Argilaga and Thea de Koning for editing the manuscript. We thank Mads Bertelsen and Hans Nieuwendijk for their scientific input. This study was funded in part by EUPRIM-NET 2, European Community grant agreement number 262443.

Authors' contributions

JB, SSA, PWK and JAML: conceived the study, participated in its design and coordination and wrote the final version of the manuscript. SR and JB:

Figure 1 Blood values of each protocol for (a) aspartate aminotransferase (AST in U L^{-1}), (b) lactate dehydrogenase (LDH in U L^{-1}), and (c) creatine kinase (CK in U L^{-1}), respectively. Data presented per individual at day 0, 1 and 2. Separate panes represent the four protocols. The four different protocols were administered to nine marmosets in a crossover design: 0.20 mg kg^{-1} meloxicam subcutaneously and 0.05 mg kg^{-1} atropine intramuscularly (IM) followed by premedication with buprenorphine (20 µg kg^{-1}; protocol BUP-A); butorphanol (0.2 mg kg^{-1}; protocol BUT-A); tramadol (1.5 mg kg^{-1}; protocol TRA-A) or no injection (control group) IM in a crossover design. After 1 hour, anaesthesia was induced by intravenous administration of 16 mg kg^{-1} alfaxalone over 90 seconds.

collected data. EJR: performed statistical analysis and interpreted the data. All authors have read and approved the final manuscript.

Conflict of interest statement

The authors confirm that they have no competing interests in the conduct of this research or preparation of this paper.

References

Bakker J, Uilenreef JJ, Pelt ER et al. (2013) Comparison of three different sedative-anaesthetic protocols (ketamine, ketamine-medetomidine and alphaxalone) in common marmosets (Callithrix jacchus). BMC Vet Res 9, 113.

Bertelsen MF, Sauer CD (2011) Alfaxalone anaesthesia in the green iguana (Iguana iguana). Vet Anaesth Analg 38, 461–466.

Crofts HS, Wilson S, Muggleton NG et al. (2001) Investigation of the sleep electrocorticogram of the common marmoset (Callithrix jacchus) using radiotelemetry. Clin Neurophysiol 112, 2265–2273.

Dahan A (2006) Opioid-induced respiratory effects: new data on buprenorphine. Palliat Med 20 (Suppl. 1), s3–8.

Flecknell P (2009) Anaesthesia of common laboratory species: special considerations. In: Laboratory Animal Anaesthesia (3rd ed.). Elsevier Science, Burliongton, MA, USA. pp. 213–223.

Giral M, Garcia-Olmo DC, Gomez-Juarez M et al. (2014) Anaesthetic effects in the ferret of alfaxalone alone and in combination with medetomidine or tramadol: a pilot study. Lab Anim 48, 313–320.

Grimm KA, Lamont LA, Tranquilli WJ et al. (2015) Veterinary Anesthesia and Analgesia (5th ed.). Iowa, Wiley Blackwell, Ames.

Grint NJ, Smith HE, Senior JM (2008) Clinical evaluation of alfaxalone in cyclodextrin for the induction of anaesthesia in rabbits. Vet Rec 163, 395–396.

Hawk CT, Leary SL, Medicine ACoLA (1999) Formulary for laboratory animals/compiled by C. Terrance Hawk, Steven L. Leary; in association with the American College of Laboratory Animal Medicine (2nd ed.). Iowa State University Press, USA, Ames.

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.

Kalchofner Guerrero KS, Reichler IM, Schwarz A et al. (2014) Alfaxalone or ketamine-medetomidine in cats undergoing ovariohysterectomy: a comparison of intra-operative parameters and post-operative pain. Vet Anaesth Analg 41, 644–653.

Keates H, Whittem T (2012) Effect of intravenous dose escalation with alfaxalone and propofol on occurrence of apnoea in the dog. Res Vet Sci 93, 904–906.

Kelly KR, Pypendop BH, Christe KL (2015) Pharmacokinetics of tramadol following intravenous and oral administration in male rhesus macaques (Macaca mulatta). J Vet Pharmacol Ther 38, 375–382.

Lau C, Ranasinghe MG, Shiels I et al. (2013) Plasma pharmacokinetics of alfaxalone after a single intraperitoneal or intravenous injection of Alfaxan((R)) in rats. J Vet Pharmacol Ther 36, 516–520.

Lenhardt R (2010) The effect of anesthesia on body temperature control. Front Biosci (Schol Ed) 2, 1145–1154.

Liguori A, Morse WH, Bergman J (1996) Respiratory effects of opioid full and partial agonists in rhesus monkeys. J Pharmacol Exp Ther 277, 462–472.

Mietsch M, Einspanier A (2015) Non-invasive blood pressure measurement: values, problems and applicability in the common marmoset (Callithrix jacchus). Lab Anim 49, 241–250.

Muir W, Lerche P, Wiese A et al. (2008) Cardiorespiratory and anesthetic effects of clinical and supraclinical doses of alfaxalone in dogs. Vet Anaesth Analg 35, 451–462.

Muir W, Lerche P, Wiese A et al. (2009) The cardiorespiratory and anesthetic effects of clinical and supraclinical doses of alfaxalone in cats. Vet Anaesth Analg 36, 42–54.

Nadeson R, Goodchild CS (2000) Antinociceptive properties of neurosteroids II. Experiments with Saffan and its components alphaxalone and alphadolone to reveal separation of anaesthetic and antinociceptive effects and the involvement of spinal cord GABA(A) receptors. Pain 88, 31–39.

Navarrete-Calvo R, Gomez-Villamandos RJ, Morgaz J et al. (2014) Cardiorespiratory, anaesthetic and recovery effects of morphine combined with medetomidine and alfaxalone in rabbits. Vet Rec 174, 95.

Pearce PC, Crofts HS, Muggleton NG et al. (1998) Concurrent monitoring of EEG and performance in the common marmoset: a methodological approach. Physiol Behav 63, 591–599.

Philippens IH, Vanwersch RA (2010) Neurofeedback training on sensorimotor rhythm in marmoset monkeys. Neuroreport 21, 328–332.

Prestes NC, Ferreire JCP, Ferraz MC et al. (2014) Cesarean sections in marmosets: white-tufted marmoset (Callithrix jacchus). Vet e Zootec 21, 92–97.

Schnell CR, Wood JM (1993) Measurement of blood pressure and heart rate by telemetry in conscious, unrestrained marmosets. Am J Physiol 264, H1509–1516.

Sessler DI (1997) Mild perioperative hypothermia. N Engl J Med 336, 1730–1737.

The Royal Society (2006) The Weatherall report on the use of nonhuman primates in research [Internet] [Cited 12/2006]. Available at: https://royalsociety.org/policy/publications/2006/weatherall-report/.

Whittem T, Pasloske KS, Heit MC et al. (2008) The pharmacokinetics and pharmacodynamics of alfaxalone in cats after single and multiple intravenous administration of Alfaxan at clinical and supraclinical doses. J Vet Pharmacol Ther 31, 571–579.

Received 27 May 2016; accepted 27 June 2017.

Available online 5 February 2018

Appendix A. Ordinal scales for quality assessment of induction and recovery

Score	Quality	Description
Induction		
1	Good	No vocalisation, salivation and compulsive licking or sneezing. No increased attention towards injection site, no involuntary and/or uncoordinated muscle activity
2	Satisfactory	Some vocalisation, some involuntary and/or uncoordinated muscle activity, salivation, compulsive licking, sneezing, some discomfort to injection site (<5 minutes)
3	Unsatisfactory	Violent struggling/no immobilisation effectuated, severe discomfort from injection (increased attention towards injection site >5 minutes), excessive salivation, vomiting, compulsive licking, sneezing, involuntary muscle activity
Recovery		
1	Good	No vocalisation, salivation and compulsive licking or sneezing, aware of environment
2	Satisfactory	Some vocalisation, salivation, compulsive licking, sneezing, some stereotypical behaviour but periods of awareness
3	Unsatisfactory	Excessive salivation, vomiting, compulsive licking, sneezing, profound stereotypical behaviour (i.e. the animals mostly moved in a circular pattern at the boundary of their enclosure while being unaware of its environment), unaware of environment

Appendix B. Scoring system for palpebral reflex, muscle tension and withdrawal reflex. Recorded at 5 minute intervals during immobilization phase in all anaesthetic treatments

Score	Quality	Description
Palpebral reflex		
0	No reflex	No narrowing of the eyelids or muscle movement
1	Moderate reflex	Delayed and/or incomplete closing of the eyelids
2	Normal reflex	The eyelids immediately close fully
Muscular tension		
0	No muscle tension	Complete relaxation, adequate muscle relaxation for performing minor invasive procedures
1	Normal muscle tension	Partial relaxation
2	Increased muscle tension	Rigidity in muscles
Pedal withdrawal reflex		
0	No reflex	No increased muscle tension and/or bending of the knee for at least one second after removing the haemostat
1	Normal reflex	There is muscle tension and/or bending of the knee
2	Increased reflex	There is increased muscle tension, bending of the knee, and muscle vibrations/involuntary movements of other limbs

