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Sedative effects of intramuscular alfaxalone in pet guinea pigs (Cavia porcellus)

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Running title: Alfaxalone in guinea pigs

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Abstract

Objective To evaluate the efficacy and side effects of alfaxalone administered intramuscularly (IM) as a sedative agent in guinea pigs undergoing survey radiographs.

Study design Prospective clinical trial.

Animals Thirty client-owned guinea pigs

Methods Following baseline assessments, 5 mg kg^{-1} alfaxalone was administered IM. Heart rate, arterial haemoglobin oxygen saturation, respiratory rate, rectal body temperature, palpebral reflex, response to toe and ear-pinch, righting reflex, posture, jaw tone, and reaction to manipulation were assessed before and after sedation, at 5-minute intervals. The time elapsed from onset of sedation to return of locomotion and coordinated limbs movements, the quality of recovery and the occurrence of undesired effects were observed and recorded.

Results The mean ± standard deviation onset of sedation was 2.7 ± 0.6 minutes. The physiological variables stayed within normal ranges until completion of the procedure. Palpebral reflex and responsiveness to both ear and toe pinch were maintained during sedation. Neither hypoxaemia nor hypothermia were observed. The duration of sedation was 29.3 ± 3.2 minutes. Sedation and recovery were uneventful and adverse effects were not observed.

Conclusion and clinical relevance In conclusion, 5 mg kg^{-1} of IM alfaxalone represents a valuable sedation protocol for healthy guinea pigs undergoing minor non-invasive procedures. Further trials are required to investigate its cardiovascular effects, its clinical usefulness in unhealthy patients and its combined use with analgesics for procedures associated with nociception.

Keywords Sedation, alfaxalone, Anaesthesia, guinea pigs

Introduction

Radiographic examination is an important diagnostic method to identify dental, gastrointestinal, respiratory, and urogenital conditions that are common in guinea pigs (*Cavia porcellus*) (Zwingerberger & Silverman 2009; Fischetti 2012). However, in order to obtain high-quality diagnostic images, sedation is frequently required to achieve immobility. Several drug combinations have been used to anaesthetize or sedate laboratory guinea pigs (Dang et al. 2008; Schmitz et al. 2016).

Nevertheless, there is a paucity of literature regarding the anaesthetic management of these small rodents in a clinical context. Short-term inhalational anaesthesia with either isoflurane or sevoflurane, as well as injectable anaesthesia achieved with α-2 agonists and ketamine, alone or in combination, have both been described to obtain diagnostic imaging in rodents and small mammals (Zwingerberger & Silverman 2009; Fischetti 2012; Hawkins & Pascoe 2012). However, none of them can be considered to be optimal for guinea pigs in terms of effectiveness, reliability, safety and reversibility. Inhalational agents may cause dose-dependent hypotension, airway irritation and, in guinea pigs, even sudden death during procedures that may result from increased adrenergic tone (Flecknell 2009; Overholser et al. 2010). Benzodiazepines result in effective sedation and immobility, but have no analgesic properties and animals can be easily aroused by nociceptive stimulation or noises (Flecknell 2009). Alpha-2-agonists have unpredictable effects in guinea pigs, when administered alone or in combination with ketamine (Richardson & Flecknell 2009). Finally, ketamine alone does result in immobilization in guinea pigs, but has been reported to cause cutaneous irritation and even muscle necrosis when administered subcutaneously or intramuscularly (IM) (Flecknell 2009; Richardson & Flecknell 2009).

Alfaxalone is a neuroactive steroid derivative of pregnanedione acting on the gamma-aminobutyric acid (GABA) receptors. Its effects on ileal GABA-induced contractions have

been previously investigated in guinea pigs (Ong et al. 1988). The new alfaxalone formulation with hydroxypropyl β cyclodextrin, licensed in many countries for intravenous (IV) use as an anaesthetic induction agent in dogs and cats, has been successfully used, alone or in combination with other drugs, in exotic captive species (Jones 2012). These include amphibians (McMillan & Leece 2011; Posner et al. 2013; Sladakovic & Robert 2014; Adami et al. 2015; Adami et al. 2016 a, b), reptiles (Bertelsen & Sauer 2011; Knotek 2014), and mammals (Marsh et al. 2009; Huyhn et al. 2015; d'Ovidio et al. 2015). Alfaxalone can also be administered IM to minimize stress associated with handling, physical examination, minor procedures (such as IV cannulation) and in fractious animals (Marsh et al. 2009; Huyhn et al. 2015; Buisman et al. 2016; Khenissi et al. 2016). At a dose of 4-6 mg kg^{-1}, IM alfaxalone produced a rapid and smooth anaesthetic induction in rabbits, followed by excellent recovery (Huyhn et al. 2015).

The purpose of this study was to evaluate the efficacy and safety of IM alfaxalone as a sedative agent in client-owned guinea pigs undergoing survey radiographs, for screening of subclinical dental and respiratory diseases. Our hypothesis was that IM alfaxalone would produce safe and adequately deep sedation in guinea pigs, suitable for short diagnostic procedures requiring immobility.

Materials and Methods

Animals

Thirty client-owned guinea pigs, belonging to the same breeder and scheduled for survey radiographs for screening of subclinical dental and respiratory diseases, were enrolled in this prospective clinical trial. Health status was assessed before sedation with physical examination (to assess general health conditions) and faecal examination (to detect subclinical intestinal parasitic infections). The study was conducted under approval of the

Clinical Research Ethical Review Board of the Royal Veterinary College (license number: URN 2016 1560), and signed informed owner consent.

Procedures

Animals were admitted 24 hours before commencing the diagnostic procedures to allow for acclimatization, and housed in groups of five littermates of the same sex in190x150x200cm cages. They were provided with pelleted guinea pig feed, ad libitum hay and water, and fresh vegetables daily. All guinea pigs were attributed an American Society of Anaesthesiologists (ASA) score based on physical and copromiscroscopic exams. Animals were not fasted before the procedure. Baseline values for heart rate (HR, from chest auscultation) pulse rate (pulse oximeter transducer), arterial haemoglobin oxygen saturation (SpO_2, from pulse oximeter transducer placed digits of pelvic limb), respiratory rate (f_R) from observation of thoracic excursion) and rectal temperature (T) were obtained in each guinea pig before sedation (T0). As a part of the baseline assessment, the following variables were also assessed in the awake animals: palpebral reflex, response to toe and ear-pinch, righting reflex, posture, jaw tone, and reaction to manipulation. The palpebral reflex was assessed with a gentle tactile stimulation of the upper eyelid; the possible outcomes were yes (present) or no (absent). The response to toe- and ear-pinch was evaluated by applying blunt surgical forceps, for a maximum of two seconds, at the level of the distal interphalangeal junction and of the base of the ear, respectively, with a score ranging from 0 to 2 where (0) indicated limb withdrawal/head movement immediately after pinching (intense response), (1) delayed limb withdrawal/head movement (more than 1 second after stimulus application), and (2) no response. The righting reflex was assessed after the guinea pigs had been placed by the observer in dorsal recumbency over a flat, firm surface. A 0-3 score was used, where (0) indicated that the animal regained sternal recumbency immediately after positioning, (1) it regained sternal recumbency within 5-10 seconds after positioning, (2) it attempted to regain

sternal recumbency but failed to achieve sternal position, and (3) it maintained dorsal recumbency with no attempts to reposition. Posture was evaluated with a score ranging from 0 to 5, as follows: (0) normal, (1) head up but sitting, (2) head down and sternal recumbency, (3) lateral recumbency, (4) dorsal recumbency but responsive to stimulation, and (5) dorsal recumbency and no response to stimulation. Jaw tone was evaluated with a 0-2 score, where 0, 1 and 2 were indicative of absent, decreased, and normal jaw tone, respectively. Finally, reaction to manipulation was assessed with a score ranging from 0 to 2, where 0 was indicative of normal reaction, 1 of decreased response, and 2 of absent response.

Following baseline assessments, 5 mg kg^{-1} (0.5 mL kg^{-1}) alfaxalone (Alfaxan 1%; Jurox, UK) was administered IM in the left or right quadriceps femoris. The animals were manually restrained during intramuscular injection. Time to onset of sedation, defined as the minutes elapsed from IM injection to lateral recumbency, was recorded. At this point, the diagnostic procedure was commenced. An electric heating pad (Eickwarm; Eickemeyer, Italy) was used to prevent hypothermia. During sedation, the same variables assessed during baseline evaluation were recorded at the following 7 time points: T5, T10, T15, T20, T25, T30 and T35, indicative of 5, 10, 15, 20, 25, 30 and 35 minutes after injection, respectively. Time to recovery, defined as the minutes elapsed from onset of sedation to return of locomotion and coordinated limb movements was recorded, as well as the duration of the clinical procedure (minutes). The time elapsed from onset of sedation to time of recovery was defined as duration of sedation. The occurrence of adverse effects, namely hypoxaemia (defined as SpO_2< 97%), severe cardiorespiratory depression (defined as decrease in the basal values for HR and f_R by 50% or more), hypothermia (T < 37.2 °C), delayed food intake (more than one hour after recovery), and gastro-intestinal disturbances observed within 72 hours of sedation, was recorded. After the end of the diagnostic procedure, the guinea pigs were allowed to recover in individual boxes in a quiet room. Fresh vegetables and drinking water were offered

at recovery and the guinea pigs were discharged from the hospital after the observation period.

Statistical analyses

Normality was assessed with the Kolmogorov-Smirnov test, and with the Shapiro-Wilk test. Data were then analysed with either one-way repeated measures analysis of variance, followed by Holm-Sidak method for multiple comparisons versus baseline (T0), or with Friedman repeated measures analysis of variance on ranks, followed by Tukey test for multiple comparisons, where it applied. The time point was used as group factor. Commercially available software was used for statistics (SigmaStat and SigmaPlot; Systat Software Inc., CA, USA). *P* values < 0.05 were considered statistically significant. Data are presented as either mean ± standard deviation or median (range), where it applies.

Results

A total of 15 male and 15 females guinea pigs that weighed 456 (320-930) grams and were 8 (3-12) months old were enrolled in the study and judged healthy based on physical and copromiscroscopic exams. All guinea pigs were assigned an ASA classification risk I. Data for HR and onset and duration of sedation and duration of the clinical procedure were normally distributed. All the guinea pigs showed a reaction to IM injection of alfaxalone, characterized by twitches of the lumbar muscles and attempts to fight physical restraint. The onset of sedation was 2.7 ± 0.6 minutes. Heart rate significantly increased after IM alfaxalone compared to baseline (from 226 ± 26 to 235 ± 30 beats minute^{-1}), and there was a statistically significant difference between the values recorded at T0 (baseline) and all the other time points except T15 (p <0.001). There were no statistically significant differences in T (which ranged between 37.2-39.5°C) and *f*R [which ranged from 85 (75-98) to 89 (78-100) breaths

minute^{-1}] between baseline and the other time points (Figs. 1−3). All the physiological variables stayed within normal ranges for the species (Flecknell 2002; Keeble 2009) until completion of the procedure. Similarly, SpO_2 remained above 96% throughout the procedure and hypoxaemia was never observed. The scores for righting reflex, reaction to manipulation and jaw tone increased, compared to baseline, during the first 15 minutes of sedation, and decreased again after T15. With respect to the aforementioned variables, there were statistically significant differences between T0 and T5, T10 and T15 ($p < 0.001$; Table 1). The scores for posture increased and remained higher than baseline values until T20 ($p < 0.001$). Palpebral reflex and responsiveness to both ear and toe pinch were maintained during sedation in all animals. In all cases, the depth and the duration of sedation (29.3 ± 3.2 minutes) achieved with IM alfaxalone were sufficient to allow completion of the clinical procedure, which lasted 30.0 ± 4.5 minutes. The survey radiographs did not reveal abnormalities in any of the guinea pigs enrolled in the trial. Sedation and recovery were uneventful and adverse effects were not observed. In addition, all animals showed normal appetite and regular defecation within one hour following the procedure.

Discussion

This study has confirmed our hypothesis that 5 mg kg^{-1} alfaxalone IM represents a valuable alternative to previously reported sedation protocols for guinea pigs undergoing minor clinical procedures (Zwingerberger & Silverman 2009; Fischetti 2012; Hawkins & Pascoe 2012). Indeed, the effects of IM alfaxalone are species-dependent. Whilst in various mammalian species it is reported to produce reliable sedation when administered either alone or in combination with other drugs (Huyhn et al. 2015; d'Ovidio et al. 2015), in chelonians it appears to be less effective (Scheelings 2013). The depth of sedation achieved in the guinea pigs enrolled in the current study was sufficient to ensure immobility, ease to positioning and

adequate muscle relaxation for at least 15-20 minutes, which is the average time required for most diagnostic studies at our institution. The minimal standard deviation also shows consistency with respect to the rapid onset of action and the duration of the sedative effect, which may indicate predictability of this sedation protocol in guinea pigs. Moreover, respiratory function and body temperature were preserved throughout the procedure, which suggests that IM alfaxalone may be a clinically useful anaesthetic choice in healthy guinea pigs.

Overall, alfaxalone provided good quality of sedation compared to other anaesthetic protocols previously described in guinea pigs. A study comparing ketamine–xylazine (administered subcutaneously, IM, or intraperitoneally), intraperitoneal pentobarbital, and IM medetomidine, found that reliable immobilization and absence of response to blood sampling were not achieved. Moreover, time to recovery ranged from 49.0 to 294.3 minutes (Dang et al. 2008). Recovery from alfaxalone sedation was smooth and uneventful, and none of the animals showed hypothermia in contrast with previous reports (Schmitz et al. 2016).

It was challenging during the current study was to distinguish between deep sedation and general anaesthesia. Alfaxalone is an induction agent capable of producing both general anaesthesia and deep sedation, depending on the dose and the route of administration. However, sedation is commonly regarded as the preferred option for non-invasive clinical procedures of short duration in small animals, and very often owners raise some concerns when general anaesthesia is proposed instead. In order to define the anaesthetic effects of IM alfaxalone, it was decided to evaluate both righting reflex and posture. In laboratory rodents, the loss of righting reflex is unarguably considered the cut off parameter between sedation and anaesthesia, as it is believed to imply unconsciousness (Flecknell 2009). Whilst certain diagnostic procedures do not necessarily require unconsciousness, other features are desirable for the purpose of obtaining good quality images, especially when procedures carrying a

potential risk for personnel safety (e.g. radiographic examinations) are to be performed. Among these features, adequate sedation and muscle relaxation are desired to achieve ease to positioning and immobility. The guinea pigs remained in lateral recumbency for a period up to 20 minutes in the absence of stimulation, but most of them tended to regain their righting reflex within 15 minutes after injection. This seems to indicate that unconsciousness - and therefore general anaesthesia - may be achieved in guinea pigs after IM alfaxalone at the dose investigated in this study, but the anaesthetic effects wear off progressively, transitioning to a state of deep sedation. Increasing the alfaxalone dose may deepen or prolong its anaesthetic effects in guinea pigs. However, investigating the effect of a higher doses was beyond the aims of this study.

Increasing the dose of IM alfaxalone would also increase the risk for cardiorespiratory side effects, as has been demonstrated in dogs, cats and rabbits (Huynh et al. 2015; Tamura et al. 2015a; Tamura et al. 2015b). Moreover, because alfaxalone is only available in Europe at a concentration of 10 mg mL^{-1} doses higher than 5 mg kg^{-1} would result in unacceptably high IM injection volumes for small rodents. It has been demonstrated that histopathological lesions of the skeletal muscles may result not only from the drug's chemical characteristics, but also from mechanical compression when large volumes are injected (Evans 2005; Thuillez et al. 2009). This represents a major limitation when dealing with smaller mammals and, as previously advocated by other investigators, the commercialization of more concentrated solutions might partially overcome this issue (Tamura et al. 2015 b).

All the guinea pigs showed a behavioural reaction to IM injection of alfaxalone. However, whether this reaction was caused by the drug itself or by the needle insertion cannot be determined. No adverse reactions (e.g. self mutilation) were noticed in the present study in the days following the procedure. However, one drawback of alfaxalone compared to other injectable agents is the lack of reversibility of the effects, as no antagonists were available at

the time of writing.

All the guinea pigs maintained unaltered response to both ear and toe pinch throughout the procedure, which indicates that analgesia was not achieved. This limits the clinical of alfaxalone as a sole agent to minor, non-invasive clinical procedures.

Although to the best of the authors' knowledge no pulse oximetry device has been validated for guinea pigs, the fact that SpO_2 remained above 96% may indicate that severe hypoxaemia did not occur despite the lack of oxygen supplementation. As rodents are predisposed to respiratory diseases, the use of anaesthetic agents with minimal impact on the respiratory function is essential.

Despite increased HR after administration of alfaxalone, the measured cardiorespiratory variables were within normal reference ranges for the species (Flecknell 2002; Keeble 2009), with no remarkable changes, in the majority of the animals enrolled. With respect to the cardiovascular function, it is worth to consider that a more comprehensive evaluation of the latter would imply at least the monitoring of the arterial blood pressure. Previous studies conducted in cats and dogs have shown that doses of alfaxalone higher than 5 mg kg^{-1} caused cardiovascular depression characterised by decreased mean arterial pressure in the absence of changes in the heart rate (Tamura et al. 2015a; Tamura et al. 2015b). Unfortunately, monitoring of systemic arterial blood pressure was not performed in this study.

The guinea pigs recruited for this study were aged 8 (3-12) months and were purchased from the same breeder. Considering that guinea pigs become sexually mature at the age of 3 months, the study population was mainly composed of adults, with a few younger animals. This may represent an unintentional selection bias, as in the present study the protocol was not tested against the neonates or geriatric animals, in which alfaxalone may have sensibly different pharmacokinetics and pharmacodynamics. Moreover, the same origin of the animals may imply a similar genetic background, an aspect which may limit the validity of our

findings to a sample poorly representative of guinea pigs in general.

In conclusion, IM alfaxalone may represent a useful means to provide deep sedation with minimal side effects to healthy guinea pigs undergoing diagnostics and minor non-invasive procedures. Further trials are required to investigate its cardiovascular effects, its clinical usefulness in unhealthy patients and its combined use with analgesics for invasive procedures. Finally, studies recruiting animals coming from different populations should be encouraged to investigate the inter-individual variability.

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Figure 2 Means and standard deviations of respiratory rates, measured by direct visualization of the thoracic excursion before and after intramuscular injection of alfaxalone, in 30 guinea pigs undergoing survey radiographic examination. T5, T10, T15, T20, T25, T30 and T35 are minutes after injection; T0 is the baseline (before injection).

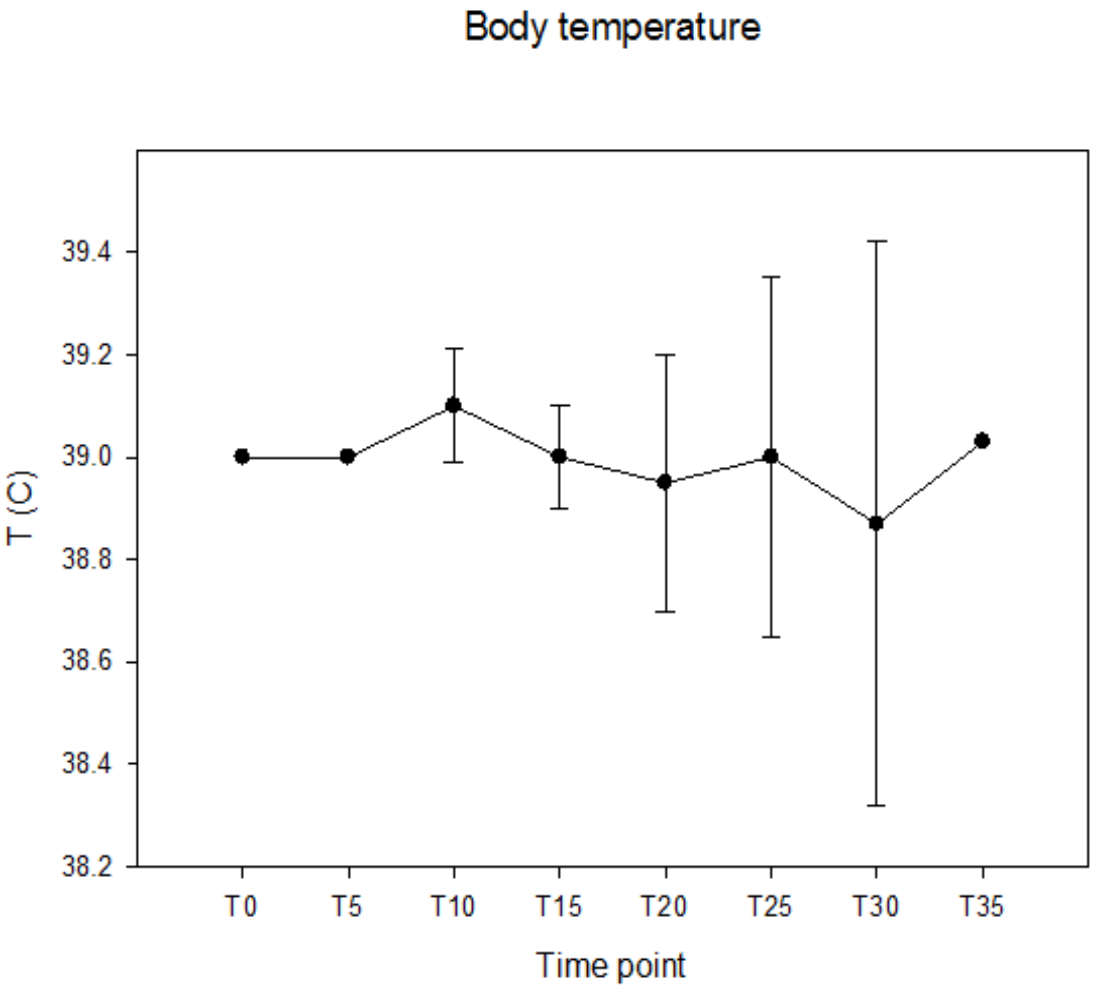
Figure 3 Means and standard deviations of heart rates, measured by thoracic auscultation before and after intramuscular injection of alfaxalone, in 30 guinea pigs undergoing survey radiographic examination. T5, T10, T15, T20, T25, T30 and T35 are minutes after injection; T0 is the baseline (before injection).

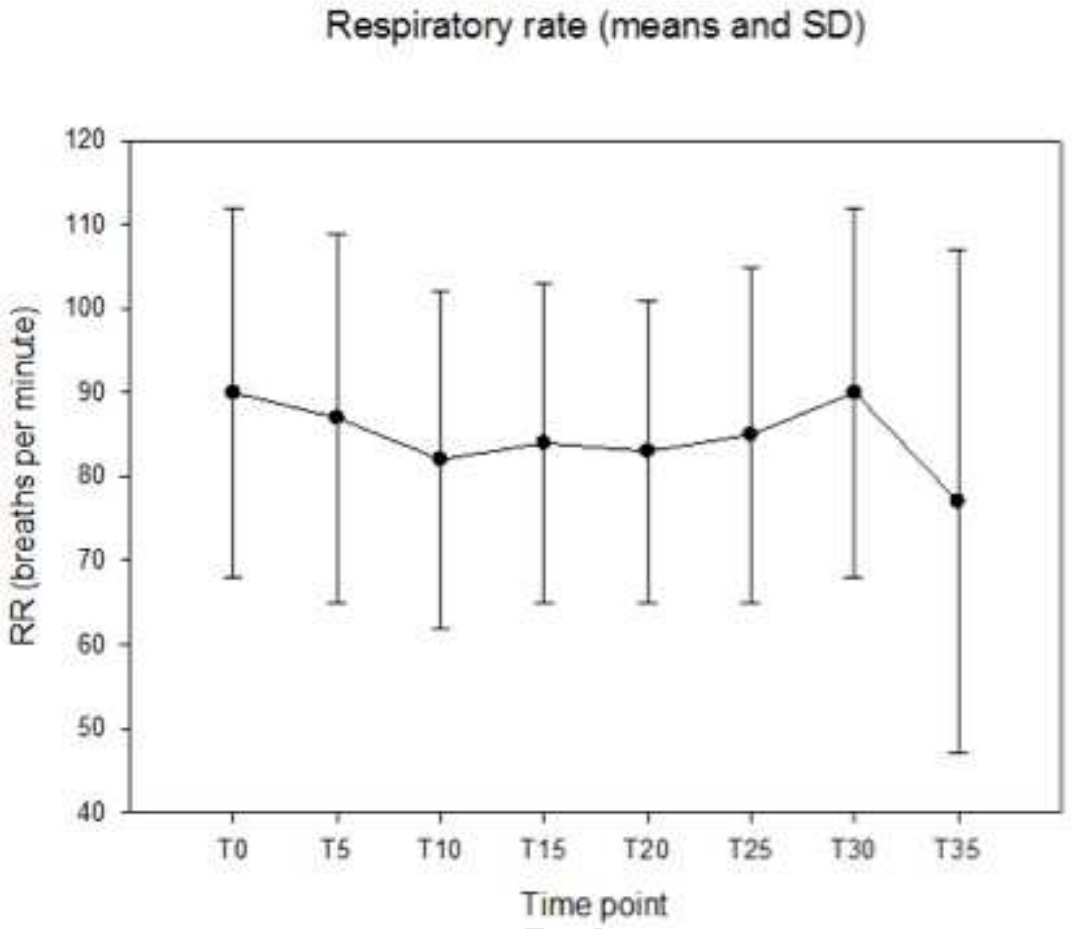
Table 1 Variables assessed during sedation with 5 mg kg^{-1} intramuscular alfaxalone in thirty guinea pigs undergoing survey radiographic exam. The time points T5, T10, T15, T20, T25, T30 and T35 are minutes after injection and *n* is the number of animals in which the clinical procedure was completed at the respective time point. Data are reported as median (range).

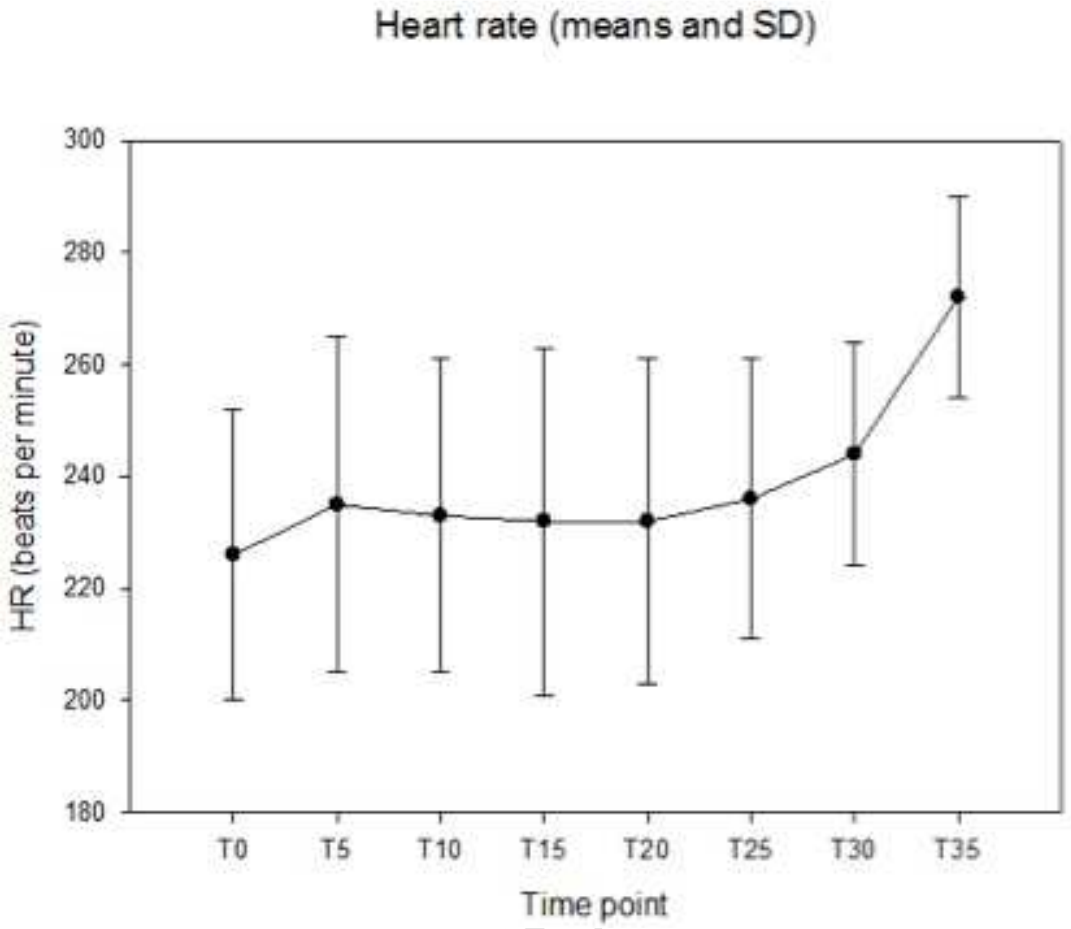
Timepoint	Righting reflex (0-3)	Reaction to manipulation (0-2)	Jaw tone (0-2)	Posture (0-5)	Palpebral reflex (yes or no)	Response to ear pinch (0-2)	Response to toe pinch (0-2)
T0 (*n*=30)	0 (0-1)	0 (0-1)	2 (1-2)	0 (0-4)	yes	0 (0-0)	0 (0-0)
T5 (*n*=30)	2 (1-2) *	2 (1-2) *	1 (1-2) *	4 (3-5) *	yes	0 (0-0)	0 (0-0)
T10 (*n*=30)	2 (1-2) *	2 (1-2) *	1 (1-1) *	4 (4-5) *	yes	0 (0-0)	0 (0-0)
T15 (*n*=30)	2 (1-2) *	2 (1-2) *	1 (0-1) *	4 (3-5) *	yes	0 (0-0)	0 (0-0)
T20 (*n*=30)	1 (1-1)	1 (1-1)	1 (1-2)	3 (3-4)	yes	0 (0-0)	0 (0-0)
T25 (*n*=27)	0 (0-1)	0 (0-1)	2 (1-2)	2 (1-3)	yes	0 (0-0)	0 (0-0)
T30 (*n*=18)	0 (0-1)	0 (0-1)	2 (1-2)	0.5 (0-1)	yes	0 (0-0)	0 (0-0)
T35 (*n*=6)	0 (0-1)	0 (0-1)	2 (1-2)	0.5 (0-1)	yes	0 (0-0)	0 (0-0)

*Significantly different from baseline (T0). Righting reflex ranged from (0) the animal regained sternal recumbency immediately after positioning, to (3) it maintained dorsal recumbency with no attempts to reposition. Reaction to manipulation ranged from (0) normal reaction to (2) absent response. Jaw tone ranged from (0) absent to (2) normal. Posture ranged from (0) normal to (5) dorsal recumbency and no response to stimulation. Response to ear pinch and toe pinch ranged from (0) intense response to (2) no response.

Figure 1: Means and standard deviations of rectal body temperature, measured before and after intramuscular injection of alfaxalone, in 30 Guinea pigs undergoing survey radiographic examination. T5, T10, T15, T20, T25, T30 and T35 are minutes after injection; T0 is the baseline (before injection).




Respiratory rate (means and SD)
RR (breaths per minute)
120
110
100
90
80
70
60
50
40
T0
T5
T10
T15
T20
T25
T30
T35
Time point


Heart rate (means and SD)
HR (beats per minute)
300
280
260
240
220
200
180
T0
T5
T10
T15
T20
T25
T30
T35
Time point