Using Alfaxalone as an Anaesthetic in Horses

Potential for improved safety for horses and handlers

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by Helen Keates, Ian Shiels, Martin Pearson, Kirby Pasloske and Wendy Goodwin

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Foreword

Anaesthesia in horses is inherently risky because of their anatomical and physiological makeup. In addition, horses by nature tend to panic if frightened, leading to injuries. Deaths and injuries in healthy horses undergoing anaesthesia for routine procedures are more common than in other domestic animals. The most common causes are cardiopulmonary disturbances and injuries sustained in induction of and recovery from anaesthesia. Thus research continues into the development of new drugs and regimens aimed at improving outcomes.

Whatever the purpose for which a horse is bred, an individual horse is likely to require anaesthesia at some time in its life. Thus, improvement in morbidity and mortality rates in equine anaesthesia is of benefit to all horse owners and managers. Many horses are of great monetary value and many have primarily sentimental value. Either way, the loss of a horse through an anaesthetic mishap is a devastating loss.

In this project, a new formulation of the steroidal anaesthetic drug alfaxalone was investigated in horses, both neonatal foals and adult horses. Results indicated that this drug is suitable for induction of anaesthesia in neonatal foals. Alfaxalone was used in combination with medetomidine to maintain anaesthesia in a group of young horses anaesthetised for castration. Conditions were satisfactory for castration and the horses had calm inductions and recoveries. Further work using techniques developed in this project could identify the mechanism by which alfaxalone causes neuroexcitation in horses recovering from long term anaesthesia so that this phenomenon can be controlled pharmacologically.

The sole manufacturer of Alfaxan® is Jurox Pty Ltd. At present, alfaxalone is available in Australia for use in cats, dogs and ‘pocket pets’ only. The results of our studies indicate that clinical trials could be used to establish if there are marketing opportunities for alfaxalone as an anaesthetic agent for use in horses, especially neonatal foals.

This project was funded from industry revenue which is matched by funds provided by the Australian Government. Jurox Pty Ltd generously performed all plasma alfaxalone assays and plasma alfaxalone data interpretation.

This report is an addition to RIRDC’s diverse range of over 2000 research publications and it forms part of our Horse R&D program, which aims to assist in developing the Australian horse industry and enhancing its export potential.

Most of RIRDC’s publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
Rural Industries Research and Development Corporation
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We are grateful to Jurox Pty Ltd for their donation of the drug Alfaxan® and for their expertise and time in analysing horse plasma samples for alfaxalone concentration.

Abbreviations

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<th>Definition</th>
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<tr>
<td>AUC(0-LLOQ)</td>
<td>area under the curve from time 0 to the last quantifiable time point</td>
</tr>
<tr>
<td>AUC(0-∞)</td>
<td>area under the curve from time 0 to infinity</td>
</tr>
<tr>
<td>AUC(％extrap)</td>
<td>area under curve percent extrapolated</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>maximum plasma concentration</td>
</tr>
<tr>
<td>Clp</td>
<td>clearance from the plasma</td>
</tr>
<tr>
<td>T ½ elim*</td>
<td>plasma elimination half-life</td>
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<td>TIVA</td>
<td>total intravenous anaesthesia</td>
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Executive Summary

What the report is about

This report describes investigations into the suitability of the neuroactive steroidal anaesthetic drug alfaxalone, formulated in hyrdoxypropyl-beta-cyclodextrin, for the induction and maintenance of anaesthesia in horses. As death and injury are not infrequent outcomes of equine anaesthesia, the search for safer anaesthetic agents is ongoing. Since it became available for use in cats and dogs in Australia in 2000, alfaxalone in hyrdoxypropyl-beta-cyclodextrin has been demonstrated to be a safe and efficacious anaesthetic agent in these species. However, little data have been published at this time of its use as an anaesthetic in horses.

Who is the report targeted at?

This report is relevant to all sectors of the equine industries. Horses, regardless of breed or purpose require anaesthesia for a variety of procedures. The loss of a horse through anaesthesia is always a significant loss, be it monetary or emotional. The outcomes from these studies will be of primary interest to veterinarians and all horse owners and equine industry members will stand to benefit from the findings.

Background

The death rate from anaesthesia in horses is high when compared with other animal species because of poor ventilation and low blood pressure during anaesthesia. Furthermore, horses frequently incur injuries during induction and recovery from general anaesthesia. Anaesthesia in young foals is particularly risky and they often require anaesthesia for a variety of procedures. Thus, there is a continuing search for safer anaesthetic drugs and techniques for equine patients. Alfaxalone dissolved in hydroxypropyl-beta-cyclodextrin was released for use in cats and dogs in Australia in 2000. Further registrations have included the UK (2007) and six other European countries (2008) through mutual recognition. Good blood pressure maintenance, good ventilation and calm inductions and recoveries have resulted in this formulation of alfaxalone capturing a large percentage of the small animal anaesthetic market in Australia and the other aforementioned countries.

The success of alfaxalone in hydroxypropyl-beta-cyclodextrin in cats and dogs and the need for better anaesthetic drugs in horses stimulated our interest in investigating its suitability for induction and maintenance of anaesthesia in horses.

Aims/objectives

The aim of this project was to test the hypothesis that alfaxalone in hydroxypropyl-beta-cyclodextrin (Alfaxan®) would provide satisfactory anaesthetic inductions, maintenance and recoveries in horses.

The specific objectives of this project were:

- To acquire basic pharmacological and pharmacokinetic data for intravenous administration of Alfaxan® in both foals and adult horses.
- To use these data and those previously established by this research group to establish a regimen for using Alfaxan® for long duration intravenous anaesthesia in the adult horse.
- To trial Alfaxan® as the sole anaesthetic agent in a clinical setting (castration of young horses in the field).
- To conduct preliminary investigations into the cause of the neuroexcitation observed in horses and other species recovering from long duration anaesthesia with Alfaxan®.
Methods used

A series of experiments was carried out in which horses, ranging in age from foals less than 12 days old to adult horses, were anaesthetised for various lengths of time. Plasma concentrations of alfaxalone were measured and correlated with depth of anaesthesia. During these experiments, measurements were made of physiological variables critical to the safety of the patient including but not limited to blood pressure, blood oxygen and carbon dioxide partial pressures.

As longer duration anaesthesia (hours) caused some excitement during the recovery of horses, preliminary investigations were conducted to establish the cause of this undesirable side effect. Three adult horses were anaesthetised with Alfaxan® for several hours whilst cerebro-spinal fluid was collected for detection of brain chemicals that could be responsible for the observed neuroexcitement. Further to this, studies have been carried out using rat hypoglossal nerve preparations to determine the effects of alfaxalone on motor neuron electrophysiological responses.

Implications for relevant stakeholders for

Anaesthesia is necessary for a variety of reasons for horses in all horse industries. As previously stated, horses are high risk anaesthetic candidates with injuries and deaths associated with anaesthesia over represented in this species. Thus, all those associated with horses, be it pleasure horses or commercial enterprise, stand to benefit from any advancement made towards safer anaesthesia for horses.

The results of our studies were consistent with benefits of alfaxalone anaesthesia as seen in other species. However, as in other species, neuroexcitation was observed in the recovery phase following longer duration anaesthesia and caution is urged here. Because of the size of the equine patient, neuroexcitation endangers both horse and handler. Therefore, it must be noted that alfaxalone is not suitable as a sole anaesthetic agent in horses. However, in combination with isoflurane anaesthesia, alfaxalone reduced isoflurane requirements and there was no neuroexcitation even following long duration anaesthesia. Once clinical studies have been completed, the techniques that we have developed will be of value to veterinarians, their clients and patients in providing improved welfare and safety outcomes for horses of all ages undergoing general anaesthesia.

The current formulation of alfaxalone, Alfaxan®, is more suited to foal anaesthesia because of the volumes required and the cost. A more concentrated solution of alfaxalone would be more suitable for use in larger horses.

Recommendations

Alfaxan®, when combined with suitable adjunctive agents, appears to be a useful short term anaesthetic agent for horses.

It is recommended that encouragement be given for the commercial development of the drug for use in this species as an anaesthetic for short procedures. This would be facilitated by re-formulation of the commercial product to a more concentrated solution for equine use.

Further studies with neonatal foals are encouraged as the results of this limited study of five foals were very promising.

Clinical trials should be conducted with alfaxalone used as a supplement to isoflurane anaesthesia following the encouraging results produced in the present study.

Further studies in horses are needed to explore the neuroexcitation reported in longer duration anaesthetics.
Introduction

Anaesthesia is routinely performed on horses to facilitate elective surgery (e.g. castration) and diagnosis and treatment of disease and injuries. Unfortunately, the risk to horses undergoing general anaesthesia is high when compared with that of other species. Johnston et al (2002) surveyed 62 clinics to find a mortality rate of 1 in 100 in over 35,000 horses undergoing anaesthesia. By comparison, the death rate in small animals is 1 in 600 (Brodbelt, 2008), and in humans, fewer than 1 in 10,000. In the survey by Johnston et al (2002) 33% of the deaths in equine patients were due to cardiovascular collapse (cardiac arrest) and 32% were due to fractures (associated with anaesthetic inductions or recoveries) or muscle damage (associated with poor perfusion of muscles in the recumbent horse). Thus, the major issues with respect to safety of horse and handler when horses are anaesthetized remain profound circulatory and ventilatory depression and pronounced ataxia on recovery.

In the horse, the transition from consciousness to recumbency, as well as the recovery from anaesthesia, poses considerable risks to personnel providing assistance to the horse. Anaesthetists must strive to achieve calm inductions and recoveries from all anaesthetics to protect the welfare of their patient and staff.

Short term anaesthesia is commonly achieved using intravenous ketamine or, in some cases, thiopentone. Ketamine is associated with increased muscle tone, rigidity and tremors. Thiopentone is associated with cardiovascular and respiratory depression.

Most long term (> 30 min) anaesthetics in horses are carried out using inhalation agents. Isoflurane is now the most commonly used inhalation agent. All inhalation anaesthetic agents are associated with some degree of cardiovascular compromise and all cause respiratory depression. Isoflurane has been shown to cause a time related decrease of cardiac index (Raisis et al, 2005).

In recent years, there has been increasing interest in the use of total intravenous anaesthesia (TIVA) in horses. This involves the administration of drugs for maintenance of anaesthesia through an intravenous infusion. Studies confirm that for long duration anaesthetics, better cardiovascular and ventilatory stability are achieved by the judicious use of intravenous anaesthetic agents in combination with sedatives than with inhalation anaesthetic agents (Luna et al, 1996, McMurphy et al, 2002).

Although ketamine is useful as an induction drug in horses, ketamine used as a sole agent, or in the absence of an adequate dose of a suitable sedative, is often associated with muscular tremors and rigidity, mydriasis, sweating and hypertension (Muir et al, 1977). Ketamine and thiopentone are unsuitable as long term anaesthetic agents because of their relatively slow metabolism, resulting in persistence of drug in plasma. As a consequence of this, recoveries become progressively longer and less satisfactory as duration of anaesthesia is increased.

The main contenders for TIVA in the horse to date have been propofol (in combination with α₂-adrenergic agonist sedatives eg xylazine, medetomidine) and ketamine (with xylazine and guiafenesin as ‘Triple Drip’).

The use of “Triple Drip” was first reported in horses by Greene et al (1986). Since then it has been used for field anaesthesia of horses for short to medium duration surgical procedures e.g. castrations and attending to wounds. It is well recognized that the longer anaesthesia is maintained with Triple Drip, the more protracted the recovery period will be with an increasing likelihood of prolonged and severe ataxia resulting in danger to both the horse and attending personnel.
Recently, propofol has been under investigation for TIVA in horses. However, in a study of horses in which anaesthesia was maintained with propofol in combination with medetomidine, 23 of 50 horses required positive pressure ventilation (Bettschart-Wolfensberger et al, 2005). This would suggest that respiratory depression is a limitation for propofol as TIVA in horses.

Alfaxalone was used in horses in the 1970s, although at the time it was licensed for use in primate and feline species only. At this time, alfaxalone was formulated with another neuro-active steroid, alphadolone and dissolved in polyoxyethylated castor oil (Cremophor®-EL), and marketed as Saffan®. Both Hall (1972) and Eales (1976) reported an unacceptable degree of neuro-excitation in horses anaesthetised with this formulation of alfaxalone. Cremophor causes allergic reactions in many species and deaths have been reported. Hence, Saffan® is no longer used in veterinary or human medicine. In the new formulation of alfaxalone, hydroxypropyl-beta-cyclodextrin is the solubilising agent. Absence of allergic reactions from alfaxalone in hydroxypropyl-beta-cyclodextrin have been demonstrated in cats and dogs (Jurox Technical Report 2000) and this has resulted in much improved cardiovascular stability. Alfaxalone causes dose dependent cardiorespiratory depression in cats and dogs; however, these effects are minimal at clinical doses (Muir et al, 2008, 2009)

In 2006, Ferré et al established the single dose pharmacokinetic profile of alfaxalone in dogs at clinical and supraclinical doses. In 2008, Ambros et al investigated alfaxalone as a possible alternative to propofol as a continuous infusion for the maintenance of anaesthesia in dogs. Respiratory depression was evident with both propofol and alfaxalone, but haemodynamic changes were minimal. Consequently, alfaxalone was considered to be suitable as an agent for maintenance of anaesthesia in dogs.

This team of researchers at the University of Queensland has been investigating Alfaxan® in horses since 2003. Our first study, a blinded cross-over study (n = 6) compared inductions and recoveries with ketamine and Alfaxan® after xylazine/guaifenesin premedication. The two anaesthetic protocols were comparable with respect to heart rate, blood pressure, haemoglobin saturation and blood lactate levels. Horses receiving ketamine had significantly lower pCO₂ and higher blood pH at times, consistent with better ventilation, but these differences were not of clinical importance. Inductions and recoveries were satisfactory with both drugs. This work was presented to an international audience at the World Congress on Veterinary Anaesthesia in Brazil in September 2006 (Keates et al, 2006).

Since the completion of this study, we have continued our research into alfaxalone in horses. We have completed a pharmacokinetic/pharmacodynamic (PK/PD) study of a single dose of 1 mgkg⁻¹ of alfaxalone following premedication with acepromazine, xylazine and guaifenesin. Inductions and recoveries were good and the monitored cardiovascular variables were satisfactory. We established mean values for plasma elimination half life (t½), plasma clearance (Clp) and volume of distribution (Vd) for alfaxalone in the horse following a single dose of alfaxalone. The rapid clearance of alfaxalone from the plasma after a single induction dose of alfaxalone indicated that alfaxalone may be suitable for delivery by infusion for long duration anaesthesia. We presented the results of this study at the World Congress on Veterinary Anaesthesia in Brazil in September 2006 (accepted for publication December, 2010, Goodwin et al).

Subsequently, we carried out a study in which anaesthesia was induced in 8 standardbred horses with Alfaxan® after premedication with acepromazine, xylazine and guaifenesin before maintaining anaesthesia with an infusion of Alfaxan® for three hours. We established a mean alfaxalone infusion rate as well as the mean alfaxalone plasma concentration from induction until the end of the 3 hour infusion, and the harmonic, mean elimination half life after the termination of the infusion. Following the cessation of the alfaxalone infusion, 6 of the 8 horses exhibited some form of excitement and hyperaesthesia that ranged from mild muscle tremors and twitching to paddling and muscle rigidity. Despite this, all horses stood without incident on the first or second attempt. However, Alfaxan® administration as a sole anaesthetic agent in horses will be viable for long procedures only if a
pharmacological regimen is developed that will abolish the neuroexcitation observed during recovery. As with all anaesthetic agents in horses, positive pressure ventilation is beneficial in long duration anaesthesia with Alfaxan® to counter respiratory depression.

The following studies were conducted to establish the safety of Alfaxan® in preparation for this RIRDC funded project:

- We demonstrated that Alfaxan® is less of a respiratory depressant than propofol in dogs (Whittem & Keates, 2011)
- We showed that Alfaxan® and ketamine are largely comparable as single dose induction agents in horses (Keates et al, 2006),
- We established the half life of alfaxalone in horses following a single bolus dose as well as the rate of plasma clearance and volume of distribution. A single bolus dose of 1 mg/kg after acepromazine, xylazine and guiafenesin gave 30 min anaesthesia. (Goodwin et al, 2010). These data enabled us to calculate an infusion rate for TIVA which we have since verified in a 4 horse pilot study (unpublished).
- We completed an 8 horse PK/PD study in which we maintained anaesthesia with Alfaxan® as a sole agent for 3 hours. In this study we collected plasma for LC-MS analysis of alfaxalone levels throughout and beyond the anaesthetic period. We also collected data on arterial blood gas tensions, blood pressure, cardiac output (thermodilution technique) and central venous blood pressure. Cardiovascular stability was good. However, elevated arterial pCO₂ values were evidence of respiratory depression. All horses recovered well although several of the horses exhibited paddling and twitching in the recovery period, before they regained consciousness. Importantly, this study demonstrated that there was no accumulation of alfaxalone over a three hour infusion (Goodwin et al, 2009).

These studies led us to consider several research questions:

- Is Alfaxan® suitable for short term anaesthesia in a) adult horses and, b) neonatal foals?
- Is Alfaxan®, in conjunction with medetomidine, suitable for long term anaesthesia in adult horses?
- Can Alfaxan® be used in conjunction with the inhaled anaesthetic agent, isoflurane, to effectively and safely reduce the requirements of isoflurane to maintain anaesthesia in horses?
- What is the mechanism responsible for the neuro-excitation seen in horses after long term (hours) Alfaxan® anaesthesia?
Objectives

The overall objective of this project was to determine the safety and efficacy of alfaxalone in hydroxypropyl-beta-cyclodextrin (Alfaxan®) as an anaesthetic agent for both short and long duration anaesthetics in horses.

The specific objectives were to:

- acquire basic pharmacodynamic and pharmacokinetic data for intravenous administration of Alfaxan® in foals
- use pharmacokinetic data previously acquired by this research group to establish an protocol for administration of Alfaxan® for long duration intravenous anaesthesia in the adult horse
- trial Alfaxan® administered as the sole anaesthetic agent for field castration of young horses
- establish techniques and conduct preliminary investigations into the cause of the neuroexcitation observed in horses recovering from long duration anaesthesia with Alfaxan®.
Methodology

Jugular vein catheterisation

A 14G 3 inch Angiocath® was placed in the left and right jugular veins after the subcutaneous deposition of 20 mg of lignocaine. The right jugular catheter was used for administering the anaesthetic agents and fluids and the left catheter was used for taking blood samples for analysis of plasma alfaxalone levels.

Carotid artery catheterisation

The horses used in the minimum alveolar concentration reduction study had previously undergone surgical elevation of the left carotid artery to a subcutaneous position. A 20G, 2-inch Teflon catheter was placed in the raised left carotid artery. This catheter was used to monitor arterial blood pressure and for sampling of arterial blood for arterial blood gas measurement.

Plasma alfaxalone estimation

Plasma samples were collected into 9 mL lithium heparin tubes and refrigerated within 20 minutes of collection. Within 3 hours of collection all samples were centrifuged for 10 minutes at 2500 rpm to separate the plasma. One mL aliquots of plasma were stored at –20°C before being relocated for storage at -70°C.

Plasma sample analysis for alfaxalone was conducted by Jurox Pty Ltd, Rutherford, NSW Australia. Samples were extracted using Waters Oasis HLB solid phase extraction (SPE) cartridges (1 cc 10 mg) with 11-hydroxy progesterone as the internal standard. The extracted equine plasma samples were analysed for alfaxalone content using Agilent 1100 series LC-MS system with electrospray ion trap mass detector. Calibration of the LC-MS was achieved using prepared standards of alfaxalone in extracted blank equine plasma. Ten (10) µL of each extracted and standard plasma samples were injected into a Phenomenex Luna C18(2) (3 µ, 3.0 x 50 mm) column using 1:1 acetonitrile water mobile phase with 1 mM ammonium acetate buffer. Alfaxalone concentration was determined by calculating the peak area ratio of the alfaxalone to 11-hydroxy progesterone. Both un-extracted and extracted samples were stored at –70°C. The lower limit of quantitation (LLOQ) of the assay was 50 µg/L. The calibration was carried out using two linear standard curves, with ranges of 50-1000 mg/L. The minimum coefficient of determination (r²) for the curve was 0.96. Both accuracy and precision were deemed acceptable if their individual residual standard deviation (RSD) was ± <20% at LLOQ or ± <15% for all other concentrations.

Pharmacokinetic analysis of data was performed using non-compartmental methods (Model: NCA 201) with uniform weighting of the data in WinNonlin Version 2.1 (Pharsight Co., CA, USA). A non-compartmental pharmacokinetic method was used for each plasma concentration-time plot for each horse. Actual doses and time points for each horse were used in the modelling. The area under the curve from the time of administration to the last quantifiable time point (AUC₀-Tₙₐₙₐ) was calculated with the linear trapezoidal rule (Gibaldi and Perrier 1982). The terminal slope (λ) was calculated by linear regression using at least the last three measured concentration points from the curve. The total AUC was calculated from the time of administration to infinity (AUC₀-∞) and represents the sum of AUC₀-Tₙₐₙₐ plus the area under the extrapolated curve. The plasma clearance (Clp), volume of distribution area (V₃D) and elimination half-life (t₁/₂ₑₐₙ₉) were calculated using standard non-compartmental formulae. The maximum plasma concentration C_max was taken directly from each individual horse’s plasma alfaxalone versus time data set.
**Depth of anaesthesia**

The depth of anaesthesia was assessed at induction and at 5-10 minute intervals by monitoring for the presence of nystagmus, eye position, anal tone, and muscle tone. Additionally, the horses’ response to a painful stimulus was assessed by using an electrical stimulus (50V, 5 Hz, 10ms, 60 sec) applied to the buccal membranes at predetermined time intervals. A positive response to stimulation was seen as purposeful movement of the horse; usually movement of the legs and/or head.

**Blood pressure measurement**

Arterial blood pressure was recorded continuously by means of a carotid artery catheter connected to a pressure transducer and the signal captured on a computer. (PowerLab, AD Instruments).

**Respiratory Blood gases**

Arterial gases and electrolytes (pH, pO2, pCO2, Na+, K+, Cl-) were measured using an IDEXX Vetstat® Analyser. Samples were collected anaerobically into 3mL heparinised syringes and stored on ice for a maximum of 1 hr before being analysed. Recordings were taken immediately after induction of anaesthesia and then at 20, 40, 60, 80, 100, 120, 140, 160, 180 and 300 minutes.

**Capnography and respiratory rate**

Gas sampled from the breathing system at the junction of the Y-piece and the patient’s endotracheal tube was analysed continuously by Datex Capnomac® or a Datex AS3® gas analysers which were calibrated before each experiment.

**Anaesthetic agent measurement**

Isoflurane levels were measured in gas sampled as above by a Datex AS3®, chosen specifically because accuracy is not affected by the presence of methane in the horse’s exhaled breath.

**Pulse oximetry and pulse rate**

Oxygen saturation of arterial haemoglobin and pulse rate were measured using a Nellcor pulse oximeter with the probe placed on the horses tongue.

**Electrocardiograph recordings**

ECG traces (Lead II) were continuously recorded and the signal captured on a computerised recording programme (Powerlab & Chart, AD Instruments).

**Ventilator settings**

Horses were ventilated to maintain pCO2 at 48.9 ± 1.1 mmHg using a Mallard Large Animal Anaesthesia Ventilator System (model 2800C-P).

**Urine output**

The urinary bladder was catheterised using an aseptic technique during the first 20 minutes of anaesthesia. Catheterisation of the bladder allowed measurement of urine output during anaesthesia and permitted continuous drainage so that urinary bladder distension during recumbency was minimised.
**Induction score criteria**

The quality of induction of anaesthesia was assessed categorically by at least three observers who then assigned a score to the procedure using the criteria below:

1. Very poor, horse fell heavily and unpredictably with rigidity and paddling and with potential to cause injury.
2. Poor, horse fell heavily and unpredictably with rigidity ± paddling.
3. Average, horse attained recumbency heavily with some rigidity ± paddling.
4. Good, horse slowly and moderately gently attained recumbency with minimal or no rigidity or paddling.
5. Excellent, horse slowly and gently attained recumbency with no rigidity or paddling.

**Recovery score criteria**

The quality of recovery from anaesthesia was assessed categorically by at least 2 observers who then assigned a numerical score using criteria below:

1. Very poor, horse attempted to stand and fell repeatedly with excitement and potential to cause injury.
2. Poor, repeated attempts to stand with some falls and excitement.
3. Average, horse stood after more than 1 attempt with knuckling and ataxia.
4. Good, horse stood on first attempt with some knuckling and ataxia.
5. Excellent, horse stood on first attempt with no knuckling and minimal ataxia.
Experimental Studies

All experimentation was done with approval of the University of Queensland Animal Ethics Committee (AEC Approval Numbers SVS/294/07/RIRDC, SVS/470/07/RIRDC, SVS/300/07/RIRDC, SVS/749/08/RIRDC, SBMS/863/08).

Long duration Alfaxan® anaesthesia in adult horses

Objective

To develop an anaesthetic protocol using Alfaxan® (alfaxalone) and medetomidine delivered as an IV infusion for use in long duration anaesthesia in adult horses.

Methods

Horses

Three standardbred horses were used in this pilot study.

Anaesthesia

Horses were premedicated intravenously (IV) with acepromazine 0.03 mg kg⁻¹ followed 20 minutes later by medetomidine 7 µg kg⁻¹ IV. Five minutes later horses received diazepam 0.05 mg kg⁻¹ (Horse 1) or guaifenesin 35 mg kg⁻¹ IV (Horse 2 and 3) followed immediately by induction with Alfaxan(R) 1 mg kg⁻¹ IV. Anaesthesia was maintained via an infusion of alfaxalone and medetomidine administered in 0.9% NaCl via a Lifecare(R) infusion pump for 110 to 180 minutes.

Medetomidine was infused at constant rate of 3.5 µg kg hr⁻¹ (Horse 1) or 5 µg kg hr⁻¹ (Horse 2 and 3). Infusion rates were altered depending on each horse’s response to electrostimulation.

Horses were placed in right lateral recumbency and the trachea intubated with a cuffed endotracheal tube. Oxygen (100%) was delivered at 6 L min⁻¹ via a circle absorber breathing system. Intermittent positive pressure (IPPV) was initiated if the horses became apnoeic (no ventilation for 60 seconds) or if end tidal CO₂ levels were above 60 mmHg.

Depth of anaesthesia was assessed by examining eye position, presence of nystagmus, presence of reflexes and general muscle tone. Electrostimulation of the buccal mucous membranes was repeated every 10 minutes. If the horse responded to electrostimulation, the alfaxalone infusion rate was increased. Conversely, if there was no response the alfaxalone infusion rate was reduced.

The following variables were measured:

- Arterial haemoglobin oxygen saturation and pulse rate
- CO₂ concentration of expired gases
- Blood pressure (invasive)
- ECG
- Respiratory rate
- Heart rate
• Mucous membrane colour.

The quality of induction of anaesthesia and recovery were scored categorically as described in 3.1.

Results

Total anaesthetic time ranged from 111 to 180 minutes. The rate of medetomidine infusion remained constant however the infusion rates of alfaxalone ranged from 1.4-3.6 mg kg hr^{-1} and are shown in Figure 1.

![Alfaxalone IV infusion rate (mg kg hr^{-1}) over time (minutes) during 110-180 minutes alfaxalone-medetomidine TIVA.](image)

The times from the end of infusion to when horses first lifted their heads, achieved sternal recumbency and stood are shown in Table 1.

<table>
<thead>
<tr>
<th>Horse</th>
<th>Extubation</th>
<th>Head lift</th>
<th>Sternal Recumbency</th>
<th>Standing</th>
</tr>
</thead>
<tbody>
<tr>
<td>111</td>
<td>6</td>
<td>25</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>121</td>
<td>4</td>
<td>Missed</td>
<td>54</td>
<td>66</td>
</tr>
<tr>
<td>180</td>
<td>5</td>
<td>15</td>
<td>26</td>
<td>34</td>
</tr>
</tbody>
</table>

Table 1  Total anaesthetic time and time (minutes) from the end of the alfaxalone-medetomidine IV infusion to; extubation, first head lift, sternal recumbency and standing.
In summary, Horses 1 and 2 had apnoeic periods, necessitating intermittent positive pressure ventilation (IPPV), and end tidal CO₂ ranged from 35-72 mmHg. Mean arterial blood pressure was well maintained and ranged from 69-124 mmHg. Heart rate was also well maintained and ranged from 28-52 beats min⁻¹. One horse (Horse 1) suffered cardiac arrhythmia from 50 to 65 minutes after induction of anaesthesia. The arrhythmia represented an atrioventricular block, an arrhythmia commonly associated with administration of an α₂ adrenergic agonist drug such as medetomidine.

Induction of anaesthesia was described as smooth and without incident in all three horses. Horse 1 had a brief period (< 60 seconds) of muscle rigidity and very mild muscle tremors immediately after induction. Horses 1 and 3 exhibited violent muscle tremors and paddling for approximately 10 minutes after the infusion was stopped. Due to safety concerns Horse 1 received 150 mg of xylazine during this period. However the drug appeared to have no effect. Horse 2 displayed mild paddling and some generalised twitching in response to tactile and auditory stimuli. Despite this, all horses stood without incident on the first attempt although Horse 1 and 3 were found to be ataxic.

Conclusion

Long duration maintenance of anaesthesia in horses using alfaxalone delivered as an IV infusion following the premedicant drugs described above resulted in an unacceptable incidence of neuro-excitation during the recovery phase. This regimen cannot be recommended for long duration anaesthesia in horses.

Pharmacodynamic and pharmacokinetic studies of Alfaxan® in neonatal foals

Objective

To acquire basic pharmacodynamic and pharmacokinetic data for intravenous administration of alfaxalone in hydroxypropyl-beta-cyclodextrin (Alfaxan®) in neonatal foals.

Methods

Horses

Five (2 female, 3 male) Australian Stock Horse foals aged 12.4±2.8 days and weighing 67.3±12.37 kg from the University of Queensland Gatton Campus commercial breeding herd were used in the study. Foals were considered healthy on the morning of the study based on results of a physical examination and haematology and biochemistry profiles.

Anaesthesia

A 2-inch 16G catheter was placed in the left jugular vein (venous blood sampling) and a 2 inch 18 G catheter placed in the right jugular vein (drug administration). Foals were premedicated with butorphanol 0.05 mgkg⁻¹ IV. After 10 minutes, alfaxalone 3 mg kg⁻¹ was administered by intravenous injection over 60 seconds.

Once recumbent, foals were placed in right lateral recumbency and the trachea was intubated. Oxygen was delivered by insufflation at 6 Lmin⁻¹. A 1 ¼ inch 22G catheter was placed in the left dorsal metatarsal artery for arterial blood pressure monitoring and blood collection for blood gas measurement.
Monitoring and sample collection

Cardiorespiratory variables and clinical signs of anaesthesia were assessed every five minutes throughout anaesthesia. Pulse rate and arterial haemoglobin oxygen saturation were evaluated with a pulse oximeter and respiratory rate was determined by counting thoracic wall excursions. Pulse quality, mucous membrane colour and capillary refill time were subjectively assessed. A continuous ECG trace, arterial blood pressure and end tidal pCO₂ were recorded. Arterial gases and electrolytes (pH, pO₂, pCO₂, Na⁺, K⁺, Cl⁻) were recorded at 5, 10, 20 and 30 minutes post induction. Blood glucose was measured prior to induction and then at 10, 20, 30 minutes and 2 hours. Nystagmus, palpebral reflex and muscle and anal tone were evaluated as indicators of anaesthetic depth. The durations of anaesthesia from induction to when the foal first lifted its head, sat on its sternum, stood and was returned to the mare were also recorded.

Time was started for the pharmacokinetic study schedule at the end of alfaxalone IV administration. At each collection time, 5 mL of venous blood was collected into lithium heparin tubes. Blood samples were collected just before dosing and at 2, 4, 6, 10, 15, 20, 30, 45, 60, 120, 240, 360 and 480 minutes. Samples were immediately stored at approximately 5 °C and within 8 hours of sampling the tubes were centrifuged (2,500 rpm, 5 minutes). The plasma was harvested and stored at −70 °C before determination of plasma alfaxalone concentrations as described in General Methods.

Statistical analysis of results

Statistical analysis of the data was performed with Graphpad Prism 5 (GraphPad Software, San Diego California USA). Data were recorded as mean ± s.d. Continuous data were compared using paired t tests or analysis of variance. Results were considered significantly different at $p < 0.05$.

Results

The maximum observed plasma concentration (Cmax) of alfaxalone occurred 2 minutes after the end of administration and was $5.54 ± 0.12$ mg L⁻¹. The harmonic, mean plasma elimination half life (t₁/₂) for alfaxalone was $22.8 ± 5.2$ minutes. The observed plasma clearance (Clp) and volume of distribution (Vd) were $20.0 ± 6.0$ mL min kg⁻¹ and $0.6 ± 0.2$ L kg⁻¹ respectively. Alfaxalone could not be detected in plasma in any of the foals after 240 minutes. A minor secondary peak in alfaxalone plasma levels was seen at 120 minutes in 2 foals (Figure 2).

![Figure 2](image)

Figure 2 Mean (SD) plasma alfaxalone concentration in foals after induction with alfaxalone 3 mg/kg IV.
Foals first moved and lifted their heads at about 20 minutes after their injection of alfaxalone and had their tracheal tubes removed at about 25 minutes. The average time after induction of anaesthesia to foals achieving sternal recumbency and standing without assistance was 35 and 37 minutes, respectively. The foals were supported until they were no longer ataxic and they were able to be returned to the mare unassisted at about 50 minutes.

Induction of anaesthesia was smooth with all foals becoming recumbent approximately three quarters of the way through the induction injection. No muscle rigidity or paddling was noted. However, two foals displayed very mild generalised muscle twitching for approximately 30 seconds after induction. Palpebral movements and nystagmus were present at times but were not consistently associated with purposeful movement.

No adverse events associated with administration of alfaxalone were observed in the study and anaesthesia was without complications. All foals breathed spontaneously throughout the anaesthetic, including the period immediately after induction. All measured variables remained within acceptable limits for anaesthetised foals except for CO₂ partial pressures which were elevated in two foals, indicating a degree of respiratory depression (Figure 3).

![Figure 3](image)

**Figure 3** Box-plot of arterial partial CO₂ pressures in 5 foals for up to 30 minutes after induction with alfaxalone (3 mg kg⁻¹) (boxes extend from 25th to 75th percentile, horizontal lines inside the box = median, bars external to box represent highest and lowest values).

**Discussion**

Baseline heart rates recorded in this study were similar to those reported by Carter, Robertson et al (1990) and Lombard, Evans et al (1984). Alfaxalone produced no significant changes in heart rate over time and no changes on the ECG, such as arrhythmias or bradycardia, were noted. Mean arterial blood pressure (MAP) measurements did not differ significantly between readings. No pre-anaesthetic measurements for MAP were available for comparison, however the recorded values were greater than those reported by Matthews, Chaffin et al (1995) for foals anaesthetised with propofol and fairly close to baseline values reported by Franco, Ousey et al. (1986) and Thomas, Maddingen et al. (1987) in neonatal foals.

Baseline respiratory rates were similar to other reported values in neonatal foals (Stewart, Rose et al. 1984; Steffey, Willis et al. 1991). Although respiratory rate decreased post induction there was no statistically significant difference from baseline values. Foals in this study exhibited mild respiratory depression evidenced by a slight hypercapnia during the first 10 minutes of anaesthesia.
Pharmacokinetics

In this study, we established the terminal elimination half life, volume of distribution and the clearance rate of alfaxalone in foals. We found a number of differences between the PK results of alfaxalone administered at 3 mg kg\(^{-1}\) in neonatal foals and the PK results from our previous study in which adult horses were administered alfaxalone at 1 mg kg\(^{-1}\) following premedication with acepromazine, xylazine and guaifenesin. The terminal half life of alfaxalone was less in foals (23 minutes) than in adult horses (33 minutes). Causes for the difference in half-life and rate of alfaxalone clearance could be related to the fact that foals have a greater proportion of total body water versus adult horses, cardiac output is greater in neonates than adults (relative to body mass) and the rate of clearance of alfaxalone in the adult horses is likely to have been influenced by the vasoactive agents acepromazine and xylazine.

Conclusion

The results of this study indicate that alfaxalone can be administered safely to foals aged less than 2 weeks at a dosage of 3 mg kg\(^{-1}\) IV following premedication with butorphanol. It provided satisfactory short term anaesthesia that would be suitable for short-term medical and surgical procedures. Recovery was rapid (head lift at 20 minutes) and complete and no detrimental effects were noted. Pharmacokinetic parameters for alfaxalone in foals were established.

A clinical trial of Alfaxan® in young adult horses anaesthetised for castration

Objective

To trial alfaxalone as the sole anaesthetic agent in a clinical setting, castration of young horses in the field.

Methods

Horses

Eleven horses; one thoroughbred cross and 10 Australian Stock Horses colts weighing 240-377 kg (mean weight 309 kg) and aged 11-30 months (mean 14.4 months) scheduled for surgical castration were used in the study. Horses were privately owned and procedures were performed with signed owner’s consent. Each Colt was considered healthy on physical examination.

Anaesthesia

The left jugular vein was catheterised. Colts were premedicated intravenously (IV) with 0.03 mg kg\(^{-1}\) acepromazine followed 20 minutes later by 7 µg kg IV\(^{-1}\) medetomidine. Five minutes later horses received guaiphenesin 35 mg kg\(^{-1}\) IV followed immediately by induction with alfaxalone at 1 mg kg\(^{-1}\) IV over a period of approximately 10 seconds. Anaesthesia was then maintained for 45 minutes via an IV infusion of alfaxalone at 2 mg kg hr\(^{-1}\) and medetomidine at 5 µg kg hr\(^{-1}\) in 0.9% NaCl administered via an infusion pump. The quality of induction and recovery were assessed using a numerical scale of 1-5 (See General Methodology).

After induction of anaesthesia, horses were placed in right lateral recumbency, the trachea was intubated and oxygen was insufflated at 15 L min\(^{-1}\). A 1 ¼ inch 20G catheter was placed in the left dorsal metatarsal artery or in the facial artery for arterial blood pressure monitoring and for blood gas measurement. Cardiorespiratory variables and clinical signs of anaesthetic depth were assessed every five minutes throughout anaesthesia. Pulse rate and arterial haemoglobin saturation were evaluated.
using a pulse oximeter and respiratory rate was determined by counting thoracic wall excursions. Pulse quality, mucous membrane colour and capillary refill time were subjectively assessed. A continuous ECG trace was recorded. Arterial blood pressure and arterial blood gases (pH, pO₂, pCO₂) and electrolytes (Na⁺, K⁺, Cl⁻) were recorded at 5, 10, 15, 20, 30 and 45 minutes post induction. Nystagmus, palpebral reflex and muscle and anal tone were evaluated as indicators of anaesthetic depth. The time intervals from induction to the following events were recorded; regaining of consciousness (purposeful movement and response to noise stimulus), first head lift, achievement of sternal recumbency and standing.

**Figure 4** Colt anaesthetised and instrumented for castration in the field.

**Surgery**

The surgical castration technique used was either a closed, open or semi-closed technique depending on the surgeon’s preference. Horses were treated with prophylactic tetanus toxoid and antitoxin injections and a 3 day course of procaine penicillin. Phenylbutazone was also given for 3 days for pain relief and to control inflammation. The initial dose of phenylbutazone was 4.4 mg kg⁻¹ IV followed by 2.2 mg kg⁻¹ orally once daily.
Figure 5  Colt undergoing castration in the field.

Statistical analysis of results

Statistical analysis was performed using Graphpad Prism 5. Continuous data were recorded as mean ± s.d and where appropriate data were analysed with paired t tests.

Results

Anaesthesia induction

Following induction of anaesthesia with 1 mg kg⁻¹ alfaxalone IV horses achieved lateral recumbency in less than 1 minute. The infusion of alfaxalone 2 mg kg hr⁻¹ and medetomidine 0.05 µg kg hr⁻¹ was started as soon as the horse was recumbent. In all 10 horses induction of anaesthesia was without incident and induction scores ranged from 4-5 (good to excellent). Additionally, in all horses ease of endotracheal intubation was scored 3 (no swallowing, intubated easily).

Infusion of alfaxalone-medetomidine and surgery

Surgery commenced at about 20 minutes after anaesthetic induction and lasted an average of 10 minutes. Most horses demonstrated brisk palpebral reflexes and intermittent nystagmus throughout anaesthesia. However this was not associated with gross purposeful movement. Surgical conditions were adequate in all cases. Two (2) horses showed positive reactions during surgery that were evidenced by slight hind leg movement or muscle tensing. Surgeons also commented on mild cremaster muscle tension with 3 horses. Total anaesthetic time in each horse was 45 minutes.
Cardiovascular effects

Cardiovascular variables are shown in Appendix Table A1. No ECG abnormalities were noted and heart rate showed no significant variations with time. However, all heart rate readings during anaesthesia did vary significantly from baseline values; which were above normal levels for resting adult horses. This may have been due to stimulus associated with the surgery. Mean arterial blood pressure (MAP) was well maintained in the normal physiological range for adult horses.

Respiration and arterial blood gases

Measurements of respiratory variables are shown in Appendix Table A2. Mean respiratory rate was lower during anaesthesia than baseline readings. Arterial blood gases showed some evidence of respiratory depression with mild elevations in arterial carbon dioxide levels however only the 15 minute reading (54.91 ± 2.25 mmHg) was significantly different to the mean 5 minute value (49.86 ± 3.035 mmHg) which was the lowest. Arterial oxygen levels were well maintained and did not vary over time.

Recoveries

On average, the horses had their tracheal tubes removed 14 minutes after cessation of the infusion and lifted their heads at 16 minutes. The times from the end of the infusion until the colts first achieved sternal recumbency and stood were, on average, 31 minutes and 37 minutes respectively. Recovery scores were 5 (excellent) in 7 horses and 4 (good) in 4 horses. All horses stood on their first attempt.

Discussion

All eleven colts castrated under an alfaxalone/medetomidine infusion experienced smooth inductions and recoveries. Conditions for intubation of the trachea were excellent for all horses. Depth of anaesthesia was appropriate for the surgical procedure with only two horses moving minimally in response to surgery. It is interesting to note that the surgeons commented on some cremaster muscle tension.

Physiological variables monitored indicated that cardiovascular stability was good. Blood pressure was well maintained with the lowest recorded mean arterial blood pressure being 71 mm Hg. No horses required pharmacological support to maintain blood pressure.

There was evidence of a degree of respiratory depression with 5 of 11 horses having paCO₂ of 60 mm Hg or greater at times during the 45 minute anaesthetic. Haemoglobin saturation readings in two horses reached 93%, indicative of mild hypoxaemia. However arterial pO₂ was in the normal range for all horses except horse 8 at 10 and 15 minutes when pO₂ was measured at 54 and 70 mm Hg. However, in this horse at these times, both the haemoglobin saturation and the mean arterial blood pressure were within the normal range.

Conclusion

Infusion of alfaxalone and medetomidine administered simultaneously as an IV infusion after acepromazine, medetomidine and guiafenesin premedication, provided satisfactory anaesthesia for 45 min characterised by smooth inductions and recoveries, good intubation conditions, good cardiovascular stability and mild respiratory depression. Satisfactory conditions for surgery were achieved.
Minimum alveolar concentration (MAC) reduction study: isoflurane administered concomitantly with alfaxalone

Objective

To establish an anaesthetic protocol for administering alfaxalone for long duration intravenous anaesthesia in the adult horse. Specifically, the objective of this study was to test the hypothesis that alfaxalone could be used as an infusion to reduce the alveolar concentration of isoflurane required to achieve a surgical plane of anaesthesia, thus achieving a reduction of the minimum alveolar concentration (MAC) value.

Methods

Study Design

The study was triple blinded and performed as a 3 treatment cross over design. Each horse received each of the 3 treatments in a randomised order with a minimum period of 21 days between each treatment.

Surgery

Six standardbred horses (3M, 3F) obtained from the University of Queensland Pinjarra Hills Research Herd, were used in the study. Previously, horses had undergone surgery for left carotid artery translocation to the subcutaneous position. Before anaesthesia horses were weighed and declared healthy based on clinical examination, haematology and biochemistry.

Catheterisation

Catheters (14G 3 inch) were placed in the left and right jugular veins. The left jugular catheter was used to administer intravenous anaesthetic agents and Hartman’s solution at 2-4 mL/kg/hr. The right jugular catheter was used to collect blood for alfaxalone plasma analysis. A 20G 2.5 inch catheter was placed in the raised carotid left artery to monitor arterial blood pressure and arterial blood gases and pH. The urinary bladder was aseptically catheterised to permit continuous drainage throughout anaesthesia and to minimise urinary bladder distension during recumbency.

Anaesthesia

The horses were premedicated with xylazine 1 mg/kg IV followed 5 minutes later by induction of anaesthesia with ketamine 2.2 mg/kg IV. They were positioned in right lateral recumbency on a 15 cm thick foam pad, the trachea intubated and anaesthesia maintained with isoflurane in oxygen delivered via a large animal circle absorber with a precision out- of circle vaporiser.

The horses were ventilated to maintain PaCO₂ between 45-55 mmHg. Mean arterial blood pressure was maintained above 60 mmHg by administering a dobutamine infusion when necessary.
Monitoring

Baseline measurements of rectal temperature, heart rate, respiratory rate, direct mean arterial blood pressure and arterial blood gases and pH were recorded prior to premedication with xylazine.

After induction of anaesthesia a continuous ECG trace was recorded. Other cardiopulmonary measurements were recorded every 10 minutes until the end of anaesthesia. These included:

- pulse oximetry and heart rate
- direct mean, systolic and diastolic arterial blood pressure
- End tidal CO₂ and inspired O₂ concentration
- Pulse quality (subjective assessment).

Body temperature was recorded every 30 min with a nasopharyngeal temperature probe. The urinary bladder was catheterised and urine output measured hourly with the aim of maintaining a urine flow of approximately 2-3 mL/kg/hr.

Arterial blood samples were collected immediately after induction and then half hourly until the end of anaesthesia so that arterial blood gases and pH (pO₂, pCO₂, pH) could be measured.

During recovery from anaesthesia the following time periods from induction of anaesthesia were recorded:

- time to endotracheal tube removal
• time to first head lift
• time to sternal recumbency
• time to standing
• scoring of recovery (previously described).

**Minimum Alveolar Concentration (MAC) determination**

Each horse was allowed to equilibrate under isoflurane anaesthesia for 90 minutes to achieve an end-tidal concentration of approximately 1.3 %. Each horse was then allocated to one of 3 treatment groups; control, low dose alfaxalone (LCRI) or high dose alfaxalone (HCRI). Horses in the control group received a bolus intravenous injection of saline followed by a saline infusion. Horses in the LCRI group received a bolus of alfaxalone (0.8 mg/kg) followed by and infusion of alfaxalone 0.6 mg/kg/hr. Horses in the HCRI group received a bolus of alfaxalone 1.1 mg/kg followed by an infusion of alfaxalone 0.9 mg/kg/hr. Thirty minutes later the isoflurane MAC determination was commenced. A noxious stimulus (50V, 5Hz, 10ms) was applied for 60 seconds to the oral mucous membranes. The delivered isoflurane concentration was increased or decreased by approximately 20% of the previous measured end-tidal concentration according to the response of the horse to an electrical stimulus. Gas was sampled and analysed continuously from the endotracheal tube deep within the horse’s thorax.

The isoflurane MAC value was defined as the average of the highest concentration allowing a positive response and the lowest concentration preventing a positive response. A positive response to electrical stimulation of the oral mucous membranes was defined as purposeful, gross movement of the limbs or head. Movements such as shivering, stiffening or changes in respiratory pattern were defined as non-purposeful movements and ignored. The measurements were made in duplicate.

**Alfaxalone infusions**

Target plasma concentrations for alfaxalone were 1.0 mg/L and 0.5 mg/L for the high and low doses respectively. Initial loading doses and infusion rates for obtaining the predetermined plasma alfaxalone concentrations were based on pharmacokinetic data for horses previously established by this research group.

Blood samples for plasma alfaxalone concentrations were obtained after the bolus administration and then 30 minutes after the start of the infusion. Additional samples were collected prior to each noxious stimulus so that the plasma alfaxalone levels could be related to the anaesthetic depth of the individual horse.

**Statistical analysis of results**

The results were expressed as mean ± SD. Normal distribution of data was verified by using the Kolgomorov-Smirnov test. Data were submitted to repeated measures analysis of variance (ANOVA) and 2-way ANOVA where appropriate. The means were compared with the Tukey or Bonferroni post test. Results were considered significantly different if $p < 0.05$ using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA).

**Results**

**Anaesthesia time and recovery**

No difference was found in anaesthetic duration between the saline and alfaxalone treatments (with high and low dose alfaxalone infusions). These data were combined for the purpose of establishing the overall mean total anaesthesia time of 304 min (range 185 – 365 min). The average recovery
times to first head lift, sternal recumbency and standing also did not differ significantly between treatment groups and are shown in Table 2.

**Table 2** Recovery times (min) after constant rate infusion of saline (control treatment), and LCRI (0.9 mg kg⁻¹ hr⁻¹) and HCRI (1.9 mg kg⁻¹ hr⁻¹) in horses anaesthetised with isoflurane.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>LCRI</th>
<th>HCRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to first head lift (minutes)</td>
<td>13 ± 15</td>
<td>20 ± 19</td>
<td>39 ± 18</td>
</tr>
<tr>
<td>Time to sternal recumbency (minutes)</td>
<td>41 ± 10</td>
<td>53 ± 14</td>
<td>63 ± 27</td>
</tr>
<tr>
<td>Time to standing (minutes)</td>
<td>47 ± 14</td>
<td>54 ± 15</td>
<td>68 ± 29</td>
</tr>
</tbody>
</table>

LCRI, low-dose constant rate infusion; HCRI, high-dose constant rate infusion. Values are expressed as mean ± SD.

Recovery scores ranged from 2 (poor) to 5 (excellent) and there was no significant difference in median scores between treatment groups. All horses recovered from anaesthesia without incident.

**MAC values**

Table 3 show the MAC values determined in this study. Values obtained for LCRI and HCRI were significantly lower than those established with a saline infusion. LCRI and HCRI decreased MAC<sub>iso</sub> by 28% and 35% respectively. The percentage decrease in MAC<sub>iso</sub> correlated to plasma alfaxalone concentrations only at the high infusion rate (Figures 7 & 8). Plasma alfaxalone concentration (mean ± SD) from the start of Alfaxan® infusion at 90 minutes was 0.6 ± 0.1 mgL⁻¹ for LCRI and 1.0 ± 0.2 mgL⁻¹ for HCRI.

**Table 3** Mean Minimum Alveolar Concentration of isoflurane in horses (±SD) after continuous saline infusion (control treatment); or Low-dose Continuous Rate Infusion (0.9 mg kg⁻¹ hr⁻¹); or High –dose Continuous Rate Infusion (1.9 mg kg⁻¹ hr⁻¹) of alfaxalone.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>LCRI</th>
<th>HCRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC (% iso)</td>
<td>0.96 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P = 0.0317</td>
<td>P = 0.0355</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAC determination time (minutes)</td>
<td>207.7 ± 86.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>217.7 ± 31.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>228.8 ± 32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

MAC, minimum alveolar concentration; LCRI, low-dose constant rate infusion; HCRI, high-dose constant rate infusion. Results are expressed as mean ± SD. Values with different letters in rows are significantly different (p < 0.05). Different lower case letters denote differences between columns and capitals denote differences between rows.
Low infusion rate:
% reduction MAC vs Alf

![Graph showing the percentage reduction of isoflurane minimum alveolar concentration (MAC) versus average alfaxalone plasma concentration (µg mL⁻¹: r = 0.07, p = 0.602). (Low Dose infusion).]

Figure 7  Percentage reduction of isoflurane minimum alveolar concentration (MAC) versus average alfaxalone plasma concentration (µg mL⁻¹: r = 0.07, p = 0.602). (Low Dose infusion).

High infusion rate:
% reduction MAC vs Alf

![Graph showing the percentage reduction of isoflurane minimum alveolar concentration (MAC) versus average alfaxalone plasma concentration (µg mL⁻¹: r = 0.80, p < 0.016). (High Dose infusion).]

Figure 8  Percentage reduction of isoflurane minimum alveolar concentration (MAC) versus average alfaxalone plasma concentration (µg mL⁻¹: r = 0.80, p < 0.016). (High Dose infusion).
Blood pressure and heart rate measurements

The mean heart rates (HR), and blood pressure measured values (mean MAP, systolic SAP and diastolic DAP) did not differ significantly between the 3 treatments and were within reference values for anaesthetised horses (Muir & Hubbell, 2009). As end-tidal isoflurane concentrations changed in response to changes in vaporiser settings as an individual horses’ MAC value was established, direct comparison of data collected from horses in each of the treatment groups at particular times was not useful. Therefore, data were compared between treatment groups at three designated stages of the anaesthetic: 1) before administration of the treatment bolus, 2) after and administration of the treatment bolus which represented the start of the infusion and, 3) at 150 minutes (approximately half of the mean anaesthetic time). Values for HR, MAP, SAP and DAP were also compared between groups and within groups at these stages. The results are presented in Table 4 and show that no significant differences existed between groups.

Dobutamine was administered in all but 1 horse at a mean starting dose of 0.33 µg kg⁻¹ minute⁻¹ for a mean time of 89 minutes per horse. As dobutamine was administered following measurement of mean blood pressure of less than 70 mm Hg, some recorded values are below 70 mm Hg. One horse in the LCRI group demonstrated tachycardia (HR range 78 to 102 beats minute⁻¹) that appeared to be associated with infusion of dobutamine and which ceased once the infusion was terminated.

Table 4 Effects of constant infusion of saline (control treatment) and LCRI (0.9 mg kg⁻¹ hr⁻¹) and HCRI (1.9 mg kg⁻¹ hr⁻¹) on heart rate and arterial blood pressure in horses anaesthetised with isoflurane at 3 specific stages of the anaesthesia.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>LCRI</th>
<th>HCRI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart Rate (beats minute⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before bolus &amp; infusion</td>
<td>35.3 ± 4.1</td>
<td>35.2 ± 5.5</td>
<td>33.8 ± 3.7</td>
</tr>
<tr>
<td>After bolus &amp; infusion</td>
<td>35.8 ± 4.7</td>
<td>42.2 ± 10</td>
<td>40.5 ± 6.8</td>
</tr>
<tr>
<td>150 minutes</td>
<td>46 ± 22.5</td>
<td>43.5 ± 7.2</td>
<td>39 ± 5.4</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before bolus &amp; infusion</td>
<td>96.8 ± 16.4</td>
<td>103.8 ± 19</td>
<td>101.3 ± 20.3</td>
</tr>
<tr>
<td>After bolus &amp; infusion</td>
<td>95.3 ± 14.3</td>
<td>117 ± 31.5</td>
<td>105.8 ± 23.5</td>
</tr>
<tr>
<td>150 minutes</td>
<td>97.7 ± 13.3</td>
<td>83.7 ± 17.6</td>
<td>98 ± 20.9</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before bolus &amp; infusion</td>
<td>54.8 ± 9.7aA</td>
<td>51.3 ± 24.5</td>
<td>60 ± 10.3</td>
</tr>
<tr>
<td>After bolus &amp; infusion</td>
<td>55 ± 6.8bA</td>
<td>71 ± 7.3</td>
<td>76.7 ± 23.4</td>
</tr>
<tr>
<td>150 minutes</td>
<td>70 ± 16.4bB</td>
<td>65.5 ± 7.1</td>
<td>67.8 ± 5.3</td>
</tr>
<tr>
<td><strong>Mean blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before bolus &amp; infusion</td>
<td>69.7 ± 8.8</td>
<td>71.5 ± 5.7a</td>
<td>72.8 ± 11</td>
</tr>
<tr>
<td>After bolus &amp; infusion</td>
<td>69.5 ± 5.4</td>
<td>87.7 ± 12.5b</td>
<td>88.5 ± 24.1</td>
</tr>
<tr>
<td>150 minutes</td>
<td>81.5 ± 15.5</td>
<td>75.5 ± 6.5a</td>
<td>77.8 ± 5.8</td>
</tr>
</tbody>
</table>

LCRI, low-dose constant rate infusion, HCRI, high-dose constant rate infusion. Before bolus & infusion, 10 minutes before IV bolus loading dose (saline, low or high dose alfaxalone); After bolus and infusion, 5 minutes after IV bolus loading dose and start of IV infusion (saline, low or high dose alfaxalone); 150 minutes; the mean time point approximately half way
through the anaesthetic. Results are expressed as mean ± SD. Values with different letters in columns are significantly different (p < 0.05). Different lower case letters denote differences between columns and capitals denote differences between rows.

**Pharyngeal temperature**

Data were unremarkable although temperatures in some horses decreased late in the anaesthetic period.

**Blood gas values**

There were no significant differences in blood gas values between treatments. Mean (±SD) for PaCO₂, PaO₂, pH for the duration of anaesthesia are shown in Appendix Table A3.

**Discussion**

The results of this study supported our hypothesis that an infusion of alfaxalone delivered concomitantly with the inhalation anaesthetic agent isoflurane would reduce the amount of isoflurane necessary to maintain anaesthesia. The MAC of isoflurane was reduced significantly by both the high and low infusion rates trialled. There was no statistically significant difference in the percentage MAC reduction between the two infusion rates of alfaxalone. This may be due to the small study sample size. Individual variation in isoflurane requirement between horses was very high at the low infusion rate. At the high infusion rate, individual variation was low suggesting that the brain concentration of alfaxalone was high enough to exert a consistent, complimentary effect to isoflurane at the level of the GABA_A receptor. However, isoflurane dose reduction was numerically but not statistically greater with the high rate of alfaxalone infusion.

Importantly, the anaesthetic regimens trialled in this study were not associated with neuroexcitation despite the long duration of the anaesthetics. Inductions were calm although some horses had poor recoveries. However, there was no significant difference in the recoveries based on treatments. Neuroexcitation was marked in horses which we anaesthetised for 2 – 3 hours with a combination of alfaxalone and medetomidine or alfaxalone as the sole maintenance agent but this was not observed in this study.

In this study, horses were administered the positive inotropic drug dobutamine so that they had mean blood pressures above 70 mm Hg. All horses but one required dobutamine. The rationale for this was that the uptake of isoflurane is affected by the perfusion of the lung tissue and therefore, hypotension of varying severity in individual horses would affect our ability to obtain meaningful MAC values. Thus, we are unable to comment on the relative effects of the three treatment regimens on arterial blood pressures.

Similarly, the respiratory stability of the horses was maintained by applying positive pressure ventilation to maintain paCO₂ < 55 mm Hg. That this was achieved is demonstrated in the table of blood gas values (Appendix Table A3). There were no significant differences between treatment groups with respect to blood gas values.

In conclusion, the results of this study support our hypothesis that alfaxalone delivered as an infusion significantly reduces the requirement of isoflurane to maintain anaesthesia in horses. Additionally when combined with isoflurane for long duration anaesthesia, alfaxalone did not result in neuroexcitation.
Investigations into the mode of action of alfaxalone in causing neuroexcitation

Objective

To establish techniques and conduct preliminary investigations into the cause of the neuroexcitation observed in horses recovering from long duration anaesthesia with alfaxalone.

Rat hypoglossal nerve preparation studies

Objective

To investigate the effect of alfaxalone on neural activity using a rat hypoglossal nerve preparation.

Methods

Juvenile male Wistar rats were euthanased. The brain stem was removed and placed in a frozen slurry of artificial cerebrospinal fluid (ACSF). At the level of the hypoglossal motor neurone, the brainstem was sliced to produce 300 µM slices. The slices were incubated at 36-37°C for 30-50 min in ACSF with MgCl₂ and CaCl₂.

The slices were then transferred to ACSF with MgCl₂ and CaCl₂ (ACSF-2Mg/1Ca) at room temperature for recording synaptic activity. The brainstem slice was placed into a well on the microscope and the hypoglossal motor neurone was located. Carbogen was bubbled through the ACSF-2Mg/1Ca which was continuously flowed through the well to nourish the brainstem slice.

A 1µm borosilicate glass electrode was used with an electrolyte solution containing ATP, GTP and Masashi’s internal cesium chloride. This electrode was whole cell clamped onto a hypoglossal motor neurone cell and synaptic current activity was recorded. With the cell stabilized, a recording could be made continuously during the experiment. DL-2-Amino-5-phosphonopentanoic acid / DL-APV / DL-2-Amino-5-phosphonovaleric acid (DL-APV, 50µM) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX, 10µM) were added to the solution to provide NMDA and non-NMDA receptor antagonist activity and allowed 6-10 minutes to act. The next solution added was alfaxalone (25µM) with DL-APV (50µM) and NBQX (10µM) for 10 minutes. Following this, the alfaxalone solution was washed off for 10 minutes with DL-APV and NBQX and then washed back to ACSF-2Ca/1Mg for a variable length of time depending on the rate of cell recovery.

Thus cell activity in the presence and absence of alfaxalone were recorded.

Results

Alfaxalone (25 µM) significantly decreased (P= 0.006, n = 8) the mean spontaneous inhibitory postsynaptic current (IPSC) frequency to 1.63 ms from 0.976 ms control. There was no significant difference in spontaneous IPSC amplitude compared with the control (P= 0.614, n=8).

These data show that alfaxalone reduces rat hypoglossal motor neurone presynaptic inhibitory transmission.

Conclusion

The technique of single cell recording has been developed and now allows the investigation of the effects of receptor stimulation and receptor blockade on signal generation in neurons. In the clinic, the neuroexcitation seen in some patients anaesthetised with alfaxalone has not been adequately controlled with adjunctive pharmacological agents. It is hoped that identifying a molecular mechanism
underlying this phenomenon will allow a rational selection of agents to be administered with alfaxalone to lessen the impact of the neuroexcitation. The demonstration of alfaxalone causing a decrease in inhibitory post-synaptic current of a motor neurone may be significant in the investigation of the mechanism of the neuroexcitation seen in many species after alfaxalone anaesthesia. Further studies are warranted.

**Equine cerebro-spinal fluid studies.**

**Objective**

To investigate the feasibility of detecting neurotransmitters in equine cerebrospinal fluid.

**Methods**

**Horses**

Three mixed breed horses (1F, 2M) aged 7-15 years and weighing 380-450 kg were used in the study. Each horse was clinically examined to ensure suitability for the trial and to record baseline parameters.

A 14G catheter was placed in the left jugular vein prior to premedication. A 20G catheter for invasive blood pressure monitoring was placed in the dorsal metatarsal artery of each horse once it was recumbent.

**Anaesthesia**

Horses were premedicated intravenously (IV) with acepromazine 0.03 mg kg\(^{-1}\) followed 20 minutes later by xylazine 1 mg kg\(^{-1}\) IV. Five minutes later horses received guaifensein 35 mg kg\(^{-1}\) IV followed immediately by induction with alfaxalone 1 mg kg\(^{-1}\) IV. Anaesthesia was maintained with an infusion of alfaxalone administered in 0.9% NaCl (5 mg mL\(^{-1}\)) via an infusion pump for 2-3 hours. The infusion rates ranged from 2.5-7 mg kg hr\(^{-1}\) varying in response to the depth of anaesthesia.

Horses were placed in right lateral recumbency and the trachea intubated with a cuffed endotracheal tube. Oxygen (100%) delivered at 6 L min\(^{-1}\) via a circle anaesthetic machine and intermittent positive pressure (IPPV) was initiated if the horses became apnoeic (no ventilation for 60 seconds) or if end tidal CO\(_2\) levels were above 60 mmHg.

**Physiological monitoring**

Baseline measurements for rectal temperature, heart rate, respiratory rate and direct mean arterial blood pressure were recorded prior to premedication with acepromazine. After induction of anaesthesia, a continuous ECG trace was recorded. Other cardiopulmonary measurements were taken every 15 minutes until the infusion stopped. These included:

- Pulse oximetry and heart rate
- Direct mean, systolic and diastolic arterial blood pressure
- End tidal CO\(_2\) and respiratory rate
- Pulse quality (subjective).
Catheterization of the cisterna magna

Approximately 40 minutes after induction of anaesthesia an indwelling atlanto-occipital subarachnoidal catheter was placed using the following method:

- The skin over the dorsal aspect of the atlanto-occipital joint (approximately 15-20 cm caudal to the ears and 8-10 cm either side of the midline) was clipped and aseptically prepared and a sterile barrier was maintained at all times. The site of catheter placement was identified and marked using a sterile pen and the horse positioned appropriately. The placement site was marked at the intersection of the cranial borders of the atlas and the external occipital protuberance (or median eminence of the nuchal crest) along the dorsal midline. The horse was positioned with the head flexed so that the median axis of the head was at 90 degrees to the median axis of the cervical spine to maximise the atlanto-occipital space. Padding was placed to ensure that the long axis of the cervical spine and head were horizontal and parallel to the ground.

- A 17G 9.84 cm Tuohy spinal needle was then inserted. With the bevel facing caudally, the needle was angled towards the lower jaw and facial crest and was advanced until the dura was penetrated at an approximate depth of 5 cm. Resistance to the advancing needle increased as the needle penetrated the nuchal ligament and then decreased resulting in a characteristic ‘pop’ as the catheter entered the cisterna magna. Correct catheter placement was verified by the presence of CSF.

- The threading assist device was then attached, the catheter was advanced to the 21 cm mark and the Tuohy needle was removed. As the needle bevel had been positioned caudally it was anticipated that the catheter was also directed caudally however this could not be confirmed. Once positioned, the excess catheter was cut and snaplock adapter fitted with a 3-way tap to facilitate sample collection. The catheter was then securely sutured to the skin using 2-0 prolene (Figure 9).

![Figure 9](image-url)  
Figure 9  Horse 3 with atlanto-occipital catheter in situ.
CSF Sample Collection and Analysis

CSF samples were collected from horses once the catheter was secured in place. One (1) mL of CSF was collected following a 2 mL discard. The samples were immediately snap frozen in dry ice and stored at – 70° degrees. Samples were collected at 60, 120, 180, 185 and 190 minutes in Horses 2 and 3. Samples were collected at 60, 120, 125 and 130 minutes in Horse 1 as the anaesthetic time had to be reduced due to failure of the alfaxalone infusion pump. Baseline samples were collected 24 hours later in the standing horse once the pharmacodynamic and pharmacokinetic effects of the anaesthesia were thought to be negligible. A baseline sample could not be obtained for Horse 2 due to catheter failure.

Samples were analysed for glycine using HPLC.

Results

Total anaesthetic time was 2 – 3 hours. The infusion rates varied from 2.5-7 mg kg hr⁻¹ (Figure 2). All horses showed post anaesthetic excitement and hyperaesthesia for up to 15 minutes after the infusion was stopped. This was demonstrated by marked muscle rigidity, twitching and paddling of the legs in response to auditory or tactile stimuli. Horse 1 showed some muscle twitching from 75-105 minutes and Horse 3 also showed intermittent muscle twitching and violent jerking for the majority of the anaesthetic period.

Respiratory rates, mean arterial blood pressure, end tidal CO₂ and ECG traces were unremarkable.

The glycine levels measured in the CSF are shown in Table 5. Baseline values are available for Horse 1 only as no sample was collected for Horse 2 and Horse 3 had glycine levels below the lower limit of quantitation (LLOQ) for the assay.

Table 5  Glycine levels (pM) in horse CSF during alfaxalone TIVA.

<table>
<thead>
<tr>
<th>Horse</th>
<th>Baseline</th>
<th>60 min</th>
<th>120 min</th>
<th>125 min</th>
<th>130 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glycine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pMol/μL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horse 1</td>
<td>Baseline</td>
<td>4.995</td>
<td>7.481</td>
<td>5.614</td>
<td>15.853</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pMol/μL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No sample</td>
<td>5.225</td>
<td>5.2</td>
<td>7.639</td>
<td>8.189</td>
</tr>
<tr>
<td>Horse 3</td>
<td>Baseline</td>
<td>&lt;LLOQ</td>
<td>4.377</td>
<td>3.099</td>
<td>4.35</td>
</tr>
</tbody>
</table>

Discussion

This study was designed to develop a technique for the collection of CSF repeatedly over a period of time and to investigate the feasibility of detecting neurotransmitters in equine CSF. These transmitters are extremely labile and therefore their measurement represented some challenge. Glycine was chosen as representative of this group of excitatory neurotransmitters as it has been extensively studied in rats.

The techniques used in this study proved to be successful in that glycine was detected in the CSF of all three horses. However, glycine levels varied widely and could not be related to observed periods of neuroexcitation. Further validation of the assay techniques is necessary. The stability of glycine in equine CSF at - 70°C must be determined.
Development of a technique for catheterising the cisterna magna to allow repeated CSF sampling lays the foundation for future studies on CSF neurotransmitter levels in horses.
Results

Basic pharmacologic and pharmacokinetic data were established for single dose intravenous administration of alfaxalone in hydroxypropyl-beta-cyclodextrin in foals. The half life of alfaxalone in foals was approximately 23 minutes compared with 33 minutes in adult horses (previously established by this research group). Foals recovered rapidly from anaesthesia and all had calm inductions and recoveries. The mean anaesthesia duration was 20 minutes with foals standing at 37 minutes and returning to the mare unassisted by 50 minutes. Anaesthesia was characterised by good cardiac stability and some minor respiratory depression.

Using pharmacokinetic data previously established by this research group, a dosing regimen was established for using alfaxalone for long duration intravenous anaesthesia in the adult horse. Alfaxalone was administered concomitantly with isoflurane resulting in a significantly reduced requirement of isoflurane to maintain anaesthesia. This was an important finding as our previous attempts to administer alfaxalone as the sole anaesthetic agent to maintain anaesthesia of long duration were associated with an unacceptable incidence of neuroexcitation in the recovery phase. When alfaxalone and isoflurane were administered concomitantly, there were no observable manifestations of neuroexcitation.

Alfaxalone and medetomidine were trialled as anaesthetic agents in a clinical setting for the castration of young horses. Anaesthesia of 45 minutes duration was associated with physiological stability and good quality inductions and recoveries. As with other anaesthetic regimens used in adult horses, the horses in this trial experienced a degree of respiratory depression evidenced by minor elevations in arterial carbon dioxide partial pressures.

We have established techniques for studying the cause of neuroexcitation in animals after alfaxalone anaesthesia. In rats, we have developed the technique of whole cell clamping onto a hypoglossal motor neurone cell so that synaptic activity can be recorded. Preliminary results showed a decrease in inhibitory post-synaptic current after exposure to alfaxalone. Elucidation of the mechanism for the observed neuroexcitation may pave the way for obtunding this response pharmacologically. In horses, we developed the technique of positioning and securing an indwelling catheter for serial collection of cerebrospinal fluid. We then demonstrated that we could detect the presence of the neurotransmitter glycine. The development of these techniques will allow further investigation into the cause of the neuroexcitation observed in horses recovering from long duration anaesthesia with alfaxalone.

The results of these studies supported the hypothesis that alfaxalone has potential application in providing safe and reliable anaesthesia in horses ranging from neonates to adults. The neuroexcitation seen in some horses undergoing prolonged alfaxalone anaesthesia was not entirely unexpected because neurosteroidal anaesthetic agents have been previously reported to cause this effect. The study identified pharmacologic agents that would lessen the severity of the neuroexcitation. Studies are continuing to identify the molecular mechanism of neuroexcitation so that more effective blocking agents can be used in the anaesthetic protocol.
Implications

Our studies have demonstrated the potential value of alfaxalone in hydroxylpropyl-beta- cyclodextrin (Alfaxan®) to the equine industries as a safe form of anaesthesia. One of the risks of horse anaesthesia is injuries to patients caused when these large animals lose consciousness or attempt to rise after surgery. Alfaxalone proved to provide very smooth inductions and recoveries; however, the agent is not without some undesirable properties. In these studies it was shown that some degree of neuroexcitation could occur, especially after very long periods of anaesthesia where alfaxalone was the sole anaesthetic agent. It must be stressed that the neuroexcitation did not adversely affect any horse and when it came time to stand all horses recovered rapidly. The results of our investigations indicate that the use of adjunctive agents may lessen the severity of this side effect. The use of alfaxalone in conjunction with isoflurane did not result in neuroexcitation.

On-going studies are attempting to identify the molecular mechanism of neuroexcitation so suitable pharmacologic agents can target this effect. Early studies are concentrating on the molecular events at the glycine receptor. Once the mechanism is identified this will have an impact for neurosteroid anaesthesia in all species.

Future research should focus on the amelioration of the neuroexcitation associated with alfaxalone in all species and identified in long duration equine anaesthetics in this study. Effective control of this side effect would offer benefits outside the horse industry including the use of neurosteroids in human anaesthesia.

The use of alfaxalone as a supplement to isoflurane offers the prospect of improved cardiovascular function compared with isoflurane anaesthesia alone. This may offer particular benefits for horses with cardiovascular compromise undergoing general anaesthesia (eg colic surgery). Further studies are needed to determine whether this is the case.

Recommendations

Publishing the results of these studies in peer-reviewed veterinary journals will facilitate adoption of the techniques by veterinarians. At this time the sole manufacturer of Alfaxan®, Jurox Pty Ltd, is targeting use in small companion animals (dogs, cats, pocket pets) because of the pricing of the agent in the marketplace.

The results of our studies indicate that there may be marketing opportunities for alfaxalone as an anaesthetic agent for use in horses, especially neonatal foals.
Appendix

Castration study

Table A1 Heart rate (HR), systolic blood pressure (SAP), mean blood pressure (MAP), diastolic blood pressure (DAP) and pulse oximetry (pulse ox).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>25 min</th>
<th>30 min</th>
<th>35 min</th>
<th>40 min</th>
<th>45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (b/min)</td>
<td>52.82 ± 7.5</td>
<td>45.18 ± 7.2a</td>
<td>44.82 ± 7.86a</td>
<td>46.27 ± 6.9b</td>
<td>46.09 ± 5.35a</td>
<td>46 ± 3.87a</td>
<td>45.82 ± 4.94a</td>
<td>46 ± 4.76a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(42-64)</td>
<td>(32-60)</td>
<td>(36-52)</td>
<td>(38-52)</td>
<td>(35-60)</td>
<td>(36-52)</td>
<td>(40-52)</td>
<td>(40-52)</td>
<td>(40-56)</td>
<td></td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>n/a</td>
<td>127 ± 15.77</td>
<td>124.2 ± 21.93</td>
<td>127 ± 18.6</td>
<td>125.5 ± 18.7</td>
<td>129.5 ± 19.98</td>
<td>121.5 ± 13.95</td>
<td>119.8 ± 18.88</td>
<td>123.5 ± 19.34</td>
<td>123.1 ± 20.13</td>
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<td></td>
<td>(103-153)</td>
<td>(84-149)</td>
<td>(94-149)</td>
<td>(94-150)</td>
<td>(100-157)</td>
<td>(100-148)</td>
<td>(96-151)</td>
<td>(93-152)</td>
<td>(98-165)</td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>n/a</td>
<td>111 ± 17.15</td>
<td>110.9 ± 20.71</td>
<td>108.8 ± 20.46</td>
<td>111.8 ± 19.12</td>
<td>115.4 ± 17.35</td>
<td>115.1 ± 17.35</td>
<td>107.2 ± 18.47</td>
<td>110.4 ± 17.85</td>
<td>109.5 ± 16.38</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>n/a</td>
<td>98 ± 5.01</td>
<td>95.64 ± 5.08</td>
<td>93 ± 5.03</td>
<td>100.1 ± 4.73</td>
<td>102.1 ± 3.49</td>
<td>104.3 ± 5.07</td>
<td>95.09 ± 4.216</td>
<td>99.64 ± 18.63</td>
<td>100.5 ± 19.71</td>
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<td></td>
<td>(79-119)</td>
<td>(65-116)</td>
<td>(61-112)</td>
<td>(62-120)</td>
<td>(85-125)</td>
<td>(82-144)</td>
<td>(76-120)</td>
<td>(74-129)</td>
<td>(69-130)</td>
<td></td>
</tr>
<tr>
<td>Pulse Ox (%)</td>
<td>95.45 ± 0.37</td>
<td>95.55 ± 1.57</td>
<td>95.36 ± 2.01</td>
<td>97 ± 0.40b</td>
<td>96.27 ± 0.38</td>
<td>97.09 ± 0.64b</td>
<td>96.82 ± 1.72b</td>
<td>97.36 ± 1.21b</td>
<td>97.45 ± 1.29b</td>
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<td>(94-97)</td>
<td>(93-99)</td>
<td>(93-99)</td>
<td>(95-99)</td>
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<td>(94-100)</td>
<td>(96-99)</td>
<td>(95-99)</td>
<td></td>
</tr>
</tbody>
</table>

* Means differ significantly from baseline (P<0.05); *b* means differ significantly from 5 minute value (P<0.05)
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>30 min</th>
<th>45 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR (br/min)</td>
<td>23.09 ± 8.36</td>
<td>8.12 ± 7.2a</td>
<td>8.1 ± 8.01a</td>
<td>7.55 ± 7a</td>
<td>8.09 ± 7.85a</td>
<td>8.46 ± 8.17a</td>
<td>8 ± 7.91a</td>
</tr>
<tr>
<td>pH</td>
<td>n/a</td>
<td>7.36 ± 0.7</td>
<td>7.34 ± 0.05</td>
<td>7.34 ± 0.07</td>
<td>7.37 ± 0.07</td>
<td>7.37 ± 0.06</td>
<td>7.37 ± 0.06</td>
</tr>
<tr>
<td>PaCO2 (mmHg)</td>
<td>n/a</td>
<td>49.86 ± 8.03</td>
<td>53.8 ± 6.36</td>
<td>54.91 ± 7.46b</td>
<td>51.73 ± 10.04</td>
<td>52.91 ± 8.51</td>
<td>56.27 ± 9.22</td>
</tr>
<tr>
<td></td>
<td>(34-57)</td>
<td>(44-61)</td>
<td>(41-66)</td>
<td>(39-68)</td>
<td>(48-75)</td>
<td>(33-66)</td>
<td>(48-75)</td>
</tr>
<tr>
<td>PaO2 (mmHg)</td>
<td>n/a</td>
<td>171.9 ± 46.45</td>
<td>142.3 ± 61.32</td>
<td>136.8 ± 64.29</td>
<td>139.9 ± 54.96</td>
<td>137.7 ± 40.68</td>
<td>117 ± 45.16</td>
</tr>
<tr>
<td></td>
<td>(108-216)</td>
<td>(54-232)</td>
<td>(48-266)</td>
<td>(53-208)</td>
<td>(69-186)</td>
<td>(50-201)</td>
<td>(50-201)</td>
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<tr>
<td>HCO2- (mmol/L)</td>
<td>n/a</td>
<td>25.8 ± 1.75</td>
<td>26.81 ± 2.21b</td>
<td>27.33 ± 1.53b</td>
<td>26.91 ± 2.30</td>
<td>27.41 ± 2.77b</td>
<td>28.36 ± 3.33b</td>
</tr>
<tr>
<td></td>
<td>(23.2-28.5)</td>
<td>(22-29.9)</td>
<td>(24-29.9)</td>
<td>(21-30.2)</td>
<td>(21.9-31.4)</td>
<td>(21.3-35)</td>
<td>(21.3-35)</td>
</tr>
</tbody>
</table>

* a Means differ significantly from baseline (P<0.05); b means differ significantly from 5 minute value (P<0.05).
MAC reduction study

Table A3  Effects of constant infusion of saline (control treatment) and LCRI (0.9 mg kg$^{-1}$ hr$^{-1}$) and HCRI (1.9 mg kg$^{-1}$ hr$^{-1}$) on PaCO$_2$, PaO$_2$ and pH in horses anaesthetised with isoflurane.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>LCRI</th>
<th>HCRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.46 ± 0.01</td>
<td>7.44 ± 0.02</td>
<td>7.44 ± 0.03</td>
</tr>
<tr>
<td>PaCO$_2$ (mmHg)</td>
<td>47.3 ± 2.6</td>
<td>51.5 ± 3.1</td>
<td>49.4 ± 4.3</td>
</tr>
<tr>
<td>PaO$_2$ (mmHg)</td>
<td>362.9 ± 60.8</td>
<td>331.8 ± 81.5</td>
<td>337.8 ± 89.7</td>
</tr>
</tbody>
</table>

LCRI, low-dose constant rate infusion, HCRI, high-dose constant rate infusion.
References


Eales, FA, 1976 Effects of Saffan administered intravenously in the horse. The Veterinary Record, 99(14) 270-2.


Whittem, T & Keates, H. ‘Dose escalation with alfaxalone or propofol in the dog and effects on respiratory depression’. Presented at ACVIM, 2011.
Using Alfaxalone as an Anaesthetic in Horses

— Potential for improved safety for horses and handlers —

by Helen Keates, Ian Shiels, Martin Pearson, Kirby Pasloske and Wendy Goodwin

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Anaesthesia in horses is inherently risky because of their anatomical and physiological makeup. In addition, horses by nature tend to panic if frightened, leading to injuries. Deaths and injuries in healthy horses undergoing anaesthesia for routine procedures are more common than in other domestic animals.

In this project, a new formulation of the steroidal anaesthetic drug alfaxalone was investigated in horses, both neonatal foals and adult horses. Alfaxalone was used in combination with medetomidine to maintain anaesthesia in a group of young horses anaesthetised for castration. Conditions were satisfactory for castration and the horses had calm inductions and recoveries.

This report is relevant to all sectors of the equine industries.

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