TITLE: Sedative and antinociceptive effects of different detomidine constant rate infusions, with or without methadone in standing horses

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Abstract

Background Equine surgery is commonly performed on standing sedated horses.

Objectives To assess sedation, antinociception and gastrointestinal motility (GIM) after a detomidine loading dose followed by 2 hours constant rate intravenous (i.v.) infusion, with or without methadone in standing horses.

Study design Seven healthy adult cross-bred horses, three geldings and four females, (404 ± 22 kg) participated in this blinded, randomized, crossover experiment.

Methods Five i.v. treatments were administered: saline (SAL), detomidine low (2.5 µg/kg bwt + 6.25 µg/kg bwt/h) (DL) and high (5 µg/kg bwt + 12.5 µg/kg bwt/h) (DH) alone or both combined with methadone (0.2 mg/kg bwt + 0.05 mg/kg bwt/h), (DLM) and (DHM), respectively. Head height above ground (HHAG), electrical (ET), thermal (TT) and mechanical (MT) nociceptive thresholds and GIM were evaluated at predetermined times between 5 and 240 minutes. Mixed effect model and Kruskal-Wallis ($p < 0.05$) were used for normal and non-normal data, respectively.

Results Sedation (<50% of basal HHAG) was achieved only for the duration of the infusion and for a further 15 minutes in DH and DHM. Nociceptive thresholds were increased above baseline, to the greatest degree and for the longest duration with DHM (ET and TT for 135 minutes and MT for 150 minutes). After DH, TT was significantly higher than baseline from 30 to 120 minutes and MT from 15 to 135 minutes. After DLH, ET was increased at 90 minutes, TT at 30 minutes and MT for 120 minutes. Intestinal motility was reduced for up to 135 minutes after DL, 150 minutes after DLM and 210 minutes after DH and DHM.

Main limitations Nociceptive thresholds are not real surgical stimuli.

Conclusion Treatment DHM produced sedation, with the most intense and consistent antinociception, with reduced GIM. Treatments DH and DLM provided comparable antinociception.

Introduction

In equine practice, surgery is often performed on standing, sedated horses, as general anaesthesia is still associated with a high mortality rate [1]. For example, procedures such as laparoscopy, dentistry, sinus trepanation and enucleation can be performed with the aid of intravenous (i.v.) sedative and analgesic constant rate infusion (CRI) combined with local anaesthesia [2,3]. Intravenous CRI aims to maintain steady plasma drug concentrations, leading to consistent sedation and analgesia. Infusions of α_2-agonists alone [4-7] or in combination with opioids [5,6,7,8] are commonly used for this purpose.

Detomidine CRI has been used under clinical conditions after loading doses of 15 [9], 10 [10,11] and 7.5 [12] µg/kg bwt in prospective studies, and of 7.5 ± 1.87 µg/kg bwt (mean ± s.d.) in a retrospective study with 51 horses [13]. These reports describe a variety of protocols with mean infusion rates varying from 6.6 to 36 µg/kg bwt/h, with surgery duration of up to 170 minutes. An in-depth literature review revealed no publications evaluating the effects of simultaneous CRIs of detomidine and an opioid such as methadone in standing horses.

Identification of a protocol that provides analgesia and an appropriate degree of sedation for standing horses is challenging. Although most of the reported protocols appear to be based on personal clinical experience, recent refinements in sequential pharmacokinetic/pharmacodynamic (PK/PD) modelling allow concentration-effect data to be determined and the effects on different PD variables [i.e. height of head above the ground (HHAG), responses to nociceptive stimuli, intestinal motility] to be predicted according to plasma concentrations [14,15]. Based on data from previous studies using a number of bolus combinations of detomidine and methadone [16], PK/PD modelling [15] was used to simulate the effects of different doses and rates of detomidine and methadone on sedation, antinociception and gastrointestinal motility .

Leading on from this, we aimed to identify a protocol under experimental conditions that provides antinociception without excessive sedation. Loading doses of detomidine at 2.5 and 5 µg/kg bwt combined with 0.2 mg/kg bwt methadone followed by infusion of detomidine (6.25 or 12.5 µg/kg bwt/h) and methadone (0.05 mg/kg bwt/h) were chosen according to the results of simulation [15,16]. Our hypothesis was that the higher doses and infusion rates of detomidine, when combined with methadone would produce antinociception with adequate.

Materials and methods

The study was designed as a randomised, placebo-controlled, observer-blinded, crossover experiment. Each horse received all treatments with a 1-week washout period between treatments. The study was performed during March and April of 2017, at the farm facilities of the School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu (Brazil) where all the horses were born and lived. Each horse was always treated at the same time of day.

Animals

Seven docile, healthy adult cross-bred horses (three geldings, four mares), 9–11 years and weighing 372–450 kg were enrolled in the study. All had been trained using positive reinforcement and used in similar antinociceptive studies. The horses were kept at pasture and brought in to covered pens with outdoor access for at least 12 h, with water ad libitum, commercial feed and hay provided until each experiment began. The animals were classified as healthy ASA I (American Society of Anesthesiologists) according to physical, musculoskeletal and lameness examination, haematology and biochemistry (complete haemogram, blood urea nitrogen, aspartate and alanine transaminases, gamma glutamyl transferase and alkaline phosphatase), performed 1–2 weeks before beginning the study. No drugs such as sedatives, analgesics, corticosteroids or nonsteroidal anti-inflammatory drugs were administered by any route for at least 1 month prior to the study.

The sample size was estimated based on the sedative (HHAG) and antinociceptive results of a pilot study [$\alpha = 0.05$, $\beta = 0.80$, 5 treatments and the differences between means and expected general s.d., (https://www.statstodo.com/SSizUnpairedDiff_Pgm.php)], and on previous data from studies in the same group of horses with similar methodology [16-18].

Study design

On the day of the experiment, each animal was weighed and fly repellent was applied to the skin. The hair over both jugular veins was clipped for catheter placement and the skin disinfected. Thereafter, 1 ml of 2% lidocaine (Cristália Produtos Químicos Farmacêuticos Ltda, São Paulo, Brazil) was injected subcutaneously and one 14-gauge catheter (G14 x 70 mm (Delta Med SRL, Viadan, Italy) was placed for drug administration.

The hair over the middle third of the dorsal aspect of both metacarpals was clipped for placement of the thermal probe (right) and mechanical actuator (left). The hair immediately proximal to the coronary band of the left thoracic limb was clipped for placement of the electrical electrodes. A strict clipping and cleaning protocol was followed in order to ensure good contact and to minimize between-electrode resistance to below 3 kiloohms (kΩ) [16,19].

Each horse was then led to the experimental room (6 m^2, without windows), previously sprayed with fly repellent. Thirty minutes were allowed for familiarization. During this period, two adhesive electrodes (2223BRQ; 3M do Brasil Ltda, São Paulo, Brazil) were placed on the prepared area at the left coronary band, separated by 8 cm and secured with adhesive strips around the hoof. A commercial horse-blanket (Topcat Metrology Ltd, Ely, UK) was placed on the horse's back and the thermal (WTT2; Topcat Metrology Ltd, Ely, UK) and mechanical (WTT1 - Topcat Metrology Ltd, Ely, UK) control units, remotely controlled with infrared signals, were attached with Velcro. The thermal probe and mechanical actuator were attached to the limbs and connected to the control units after the horse was placed in the restraining stocks. A blood pressure cuff (DURA-CUF CRITIKON 17–25 cm; GE Healthcare, Helsinki, Finland) was placed at the base of the tail to measure heart rate (HR) and systolic arterial blood pressure (SAP) using a non invasive Doppler system (Model 812 - Parks Medical Electronics, Inc., Aloha, Oregon, USA).

The five treatments were all comprised of a bolus injection followed by a 2 hour CRI, assigned using a randomization website (https://sorteador.com.br). The treatments were: saline (SAL) (Fresenius Kabi, São Paulo, Brazil), detomidine low dose (DL) (2.5 µg/kg bwt detomidine; Ourofino Saúde Animal, São Paulo, Brazil; followed by a CRI of 6.25 µg/kg bwt/h), detomidine high dose (DH) (5 µg/kg bwt detomidine; followed by a CRI of 12.5 µg/kg bwt/h), detomidine low dose with methadone (DLM) (2.5 µg/kg bwt detomidine + 0.2 mg/kg bwt methadone; Cristália Produtos Químicos e Farmacêuticos Ltda, São Paulo, Brazil; followed by 2 CRIs, detomidine 6.25 µg/kg bwt/h + methadone 0.05 mg/kg bwt/h), and detomidine high dose with methadone (DHM) (5 µg/kg bwt detomidine + 0.2 mg/kg bwt methadone; followed by 2 CRIs, detomidine 12.5 µg/kg bwt/h + methadone 0.05 mg/kg bwt/h). All bolus doses and infusions were diluted by an assistant investigator (M.W.F.) with saline to 10 and 18 ml, respectively, in order to keep the main investigator blinded. The CRIs were delivered by two calibrated syringe drivers

(DigiPump SR8x;Digicare Biomedical Technology Inc, Boynton Beach, Florida, USA; and Pilot Anestesia; Fresenius Vial, Brezins, France).

Sedation and responses to noxious stimuli were evaluated in triplicate for baseline values before drug administration (T0), and once only at T5 (5 minutes after drug administration), T15, T30, T60, T90, T120, T135, T150, T180, T210 and T240. Intestinal motility was scored at the same time points starting at T15. Evaluation of the variables is described elsewhere [16] and summarised briefly below:

Sedation

The sedation variables were always evaluated by a single investigator (A.R.O.) who was unaware of the treatment identity. To evaluate the degree of sedation, the height above the ground of the lower lip (HHAG) was measured against a scale on the wall [5,6]. Quality of sedation was scored using numerical rating scales (NRS) evaluating the degree of ataxia as well as responses to tactile, acoustic and visual stimuli, always in this order. Ataxia and response to stimulus were scored from 0 to 3 (0 no ataxia, no response; 3 maximal ataxia, maximal response) (Appendix A).

Nociceptive threshold testing

Nociceptive stimuli were applied at each time point immediately after the sedation scoring, and the response were all evaluated by the main investigator (M.G.M), also blinded to the treatment identity. Stimuli were always applied in the same order: electrical, thermal, mechanical. Aversive reactions were considered positive responses when the horse lifted its foot, pawed the ground, stamped, flexed the limb or walked to avoid the stimulus [20].

Electrical threshold testing

Before each electrical stimulus was applied, a digital multimeter (XX) was used to confirm that the resistance between electrodes was < 3 kΩ [19]. The stimulus consisted of a pulsatile current of 10 millisecond square waves (ms) at 10 Hertz (Hz), delivered by an electrical stimulator. The voltage, initiated at 1 Volt (V), was increased in steps of 1 V every 5 seconds and was stopped immediately after an avoidance response was seen or the voltage reached 20 V.

Thermal threshold testing

A thermal probe with a heating element (probe 3) [21], connected to the thermal control unit, was placed on the clipped area of the dorsal aspect of the right metacarpus and attached with an elasticated band secured by Velcro. The skin temperature was recorded in degrees Celsius (°C) after at least 5 minutes equilibration. The ramped stimulus (heating at 0.8 °C/s) was then applied via the automatic wireless control system until a positive response was observed or the cut-out at 60 °C reached. In order to avoid focal tissue damage, the probe was moved 1-2 cm proximally on the limb after each stimulus, independent of the temperature reached [16].

Mechanical threshold testing

A pneumatic actuator with a 1 mm round-ended pin [22] was held with a brushing boot on the clipped area of the dorsal aspect of the left metacarpal area (mid bone), tensioned against the leg with an elasticated band secured with Velcro and connected to the control unit with non-distensible tubing. The force in Newtons (N) of the pin pressing on the skin surface was increased at 0.8 N/s by the automatic wireless control system. The stimulus was stopped when an aversive response was observed or the cut-out value of 20 N reached.

Cardiopulmonary variables

Each SAP value was corrected according to the height difference between the shoulder joint and the cuff, taking the shoulder joint to represent the level of the right atrium of the heart. A height difference of 10.2 cm was considered to be equivalent to 7.5 mmHg. The respiratory rate (RR) was measured by observation of chest movements over 15 seconds.

Abdominal auscultation

The main investigator (M.G.M) auscultated each of the four abdominal quadrants (right dorsal, right ventral, left dorsal and left ventral) for 1 minute each and awarded a motility score, from 0 to 5 according to Boscan *et al.* (2006) [23]. The sum of the scores was recorded, ranging from 0 to 20 (0 no motility, 20 maximal motility possible).

Statistical Analysis

For each variable, normality was assessed graphically and with normality tests (Shapiro-Wilk). Descriptive measurements were generated. For parametric variables, the mixed

model ANOVA with Tukey's post-hoc test was used to evaluate the differences between treatments and time points; data are shown as mean ± s.d. For non-parametric variables, Friedman's test with Dunn's post-hoc test was used; data are shown as median (range). A significance level less than 0.05 was adopted for all analysis and the calculations were made with the aid of Statistical Analysis Software - SAS version 9.4.

Results

All the horses received all treatments and completed the study without complications. They ate and defecated normally at the end of each session, without signs of abdominal discomfort.

Sedation

Sufficient sedation (< 50% of basal HHAG) [6] was only achieved for the duration of the infusion and for 15 minutes more in DH and DHM. Significant differences within and between treatments are shown in Figure 1.

Ataxia scores were significantly higher than baseline [0 (0 – 0)] after DHM at T30 [2 (1 – 3)] and T60 [2.5 (1 – 3)]. Comparing treatments, ataxia was more pronounced after DHM than SAL at T15 [2 (1 – 3)] *versus* [0 (0 – 1)], and at T30 [2 (1 – 3)], T60 [2.5 (1.5 – 3)], T90 [1.5 (1 – 3)] and T120 [2 (1 – 3)] *versus* [0 (0 – 0)], and than DL at T15 [2 (1 – 3)] *versus* [0 (0 – 1)]. Scores for responses to tactile stimuli were lower at T5 than SAL [3 (2 – 3)] after DHM [1 (0 – 3)], at T30 [3 (2 – 3)] *versus* [0.5 (0 – 2)], at T60 [3 (2 – 3)] *versus* [1 (0 – 3)] and at T90 [3 (1 – 3)] *versus* [1.5 (0 – 3)]. Scores for responses to tactile stimuli were lower than baseline [2 (1 – 3)] only for treatment DL at T30 and at T60 [0.5 (0 – 1)]. Between treatments, scores were lower at T60 after DL [0.5 (0 – 1)] than SAL [2 (1 – 2)].

Nociceptive threshold testing

Treatment DHM resulted in the highest thresholds for all the 3 stimuli as well as being responsible for most of the cut-out values reached for the thermal and mechanical modalities: nociceptive thresholds increased above baseline, to the greatest degree and for the longest duration (electrical and thermal for 135 minutes and mechanical for 150 minutes). Treatments DH and DLM provided comparable antinociception. Significant

differences within and between treatments are shown in Figures 2, 3 and 4 for electrical, thermal and mechanical thresholds, respectively.

Abdominal auscultation

Intestinal motility was reduced for up to 135 minutes after DL, 150 minutes after DLM and 210 minutes after DH and DHM. Significant differences within and between treatments are shown in Figure 5.

Throughout the study period, cardiovascular function was maintained well within acceptable limits in all horses undergoing all treatments, with no differences from baseline and between treatments for HR, and only one single difference at one time point from baseline for SAP (Table 2). All treatments produced a reduction in the RR that persisted for 60 and 90 minutes following the end of infusion of detomidine low and high doses, respectively.

One horse overreacted with mild 'head shaking' to acoustic and visual stimuli for up to T30 when receiving treatment DLM. No other complications were observed.

Discussion

This study indicates that the higher detomidine dose combined with methadone (treatment DHM) produced the most intense and consistent antinociception of all the protocols for the three stimuli. Sedation and effects on intestinal motility were similar to the high dose of detomidine alone. Low doses of detomidine with or without methadone produced less antinociception and sedation but still reduced intestinal motility for the duration of the infusion, although normal scores returned faster.

According to our results, treatment DHM produced the most intense antinociceptive effects and these were maintained during the whole 2-hours infusion period. The low dose of detomidine did not produce any antinociception, however when methadone was included, antinociception was mild and similar to that observed with the high dose of detomidine alone, showing that methadone enhanced the detomidine-induced antinociception in a similar manner to that with detomidine *boli* of 5 [16] and 10 µg/kg bwt [17,18].

Sedation indicated by more than or equal to a 50 % reduction in HHAG was similar to that observed after a bolus of 5 µg/kg bwt of detomidine as reported previously

[16], and described as 'sufficient' sedation [6]. According to this criteria, overall, only horses treated with the high dose of detomidine were 'sufficiently' sedated. In contrast, low doses of detomidine, regardless of the inclusion of methadone, produced less sedation. This is important if these protocols are used in horses undergoing standing surgery when an adequate degree of sedation is essential. With regard to the quality of sedation, some degree of ataxia and reduction in the responses to stimuli are inevitable when a high dose and infusion rate of detomidine are used in combination with methadone.

Cardiovascular function remained within clinically acceptable limits. The low dose of detomidine kept the cardiovascular effects of this drug to a minimum [16]. A reduction in the RR was noted in all treated horses to a similar degree, and lasted throughout the two hours infusion, with slight improvement towards the end of the experiment. Reductions in RR occurred when the drugs were administered as single *boli*, alone or in combination [16].

All treatments reduced intestinal motility; the higher doses of detomidine produced the greatest effects and for the longest period after the end of the infusion. Similar results were also observed when these drugs were given as *boli* [16], as well as by simulation based on PK/PD models [15]. Although no signs of colic were observed, the reduction in the motility scores suggests that horses would benefit from a close follow-up after clinical treatment during the first 12 – 24 hours.

Taking into account the above considerations, the combination of methadone and the highest dose of detomidine appears to be the most promising treatment for more invasive surgical procedures. Detomidine at 5 µg/kg bwt with 0.2 mg/kg bwt of methadone also provided antinociception with adequate sedation in a previous report [16]. For prolonged maintenance of sedation and antinociception, the 12.5 µg/kg bwt/h rate for detomidine co-administered with methadone appeared to be the best of all the treatments studied here. When given alone, detomidine infusion rates of around 9 µg/kg bwt/h were successfully used for laparoscopy [9,10], whereas rates of approximately 20 µg/kg bwt/h were required for dental or sinus procedures [11,12]. More clinical studies are needed to determine if the DHM protocol might be useful, or if the rate should be adapted to different surgical procedures.

We used the synthetic opioid methadone in order to enhance the sedative properties of detomidine, to provide analgesia [24], and to reduce the doses and infusion rates of the α_2-agonist. To date, the use of methadone as a co-infusion with an α_2-agonist

has not been reported in horses. Excitatory effects, such as head shaking, linked to the use of 0.2 mg/kg bwt of methadone [17] may be present, especially when using low doses of detomidine. In the present study, only one horse showed mild 'head The κ- agonist butorphanol is the opioid that has been the most widely studied for use in a CRI [5-8], but methadone, as a full μ-agonist, might be more appropriate in more painful surgeries. Two reports suggest that buprenorphine, a partial μ-agonist opioid, when combined with detomidine infusion, although contributing to antinociception, may lead to abdominal pain and increase locomotor activity [11,12]. However, minimal complications were observed in a large clinical study in horses sedated with romifidine and buprenorphine [25].

Most of the protocols used for standing sedation in horses are based on clinical experience, experimental [4-10], clinical [11,12] or retrospective [13] studies. Our study is innovative in equine research as we used the data from our previous *boli* study [16], to perform PK/PD modelling [15] and set dose/rates ranges that would allow different degrees of sedation and antinociception for this experimental study. Therefore, modelling will allow us to predict more precisely the expected effects of future clinical trials.

The main limitation of our study is the extrapolation of the results to a clinical scenario as: (i) nociceptive thresholds are not real surgical stimuli, even when validated for experimental studies in standing horses [20]; (ii) surgical pain in different locations may differ in intensity due to specific innervation; and (iii) understanding of the magnitude of inter-animal variability is limited from a group of 7 horses. Study of a larger clinical population undergoing a range of standing surgical procedures is needed to confirm the predicted clinical effects.

Conclusions

In conclusion, the treatment with the highest dose of detomidine combined with methadone produced the most intense and consistent antinociception, with minimal adverse effects. This protocol might be useful for clinical surgical procedures. Future studies are justified to evaluate its clinical applicability in standing horses.

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