RESEARCH PAPER

LL de Carvalho et al.

Xylazine–opioid combinations in sheep

Sedative and cardiopulmonary effects of xylazine alone or in combination with methadone, morphine or tramadol in sheep

Leonardo L Carvalho*, Lilian T Nishimura*, Luisa P B Borges*, Sofia A Cerejo†, Isadora O J Villela*, Adam Auckburally‡ & Ewaldo de Mattos-Junior*

*School of Veterinary Medicine, University of Franca, Franca, SP, Brazil

†School of Veterinary Medicine and Animal Science, São Paulo State University (Universidade Estadual Paulista, UNESP) Botucatu, Botucatu, SP, Brazil

‡Department of Small Animal Clinical Sciences, School of Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

Correspondence: Ewaldo de Mattos-Junior, Faculdade de Medicina Veterinária, Universidade de Franca, Avenida Dr Armando Salles de Oliveira, 201, Parque Universitário, Franca, SP 14404-600, Brazil. E-mail: ewaldomattos@hotmail.com

Abstract

Objective To evaluate the cardiopulmonary and sedative effects of xylazine alone or in combination with methadone, morphine or tramadol in sheep.

Study design Experimental, prospective, crossover, randomized, blinded study.

Animals Six Santa Inês breed sheep (females) aged 12 ± 8 months and weighing 39.5 ± 7.4 kg.

Methods Sheep were sedated with each of four treatments in a randomized, crossover design, with a minimum washout period of 7 days between treatments. Treatments were: X [xylazine (0.1 mg kg^{-1})]; XM [xylazine (0.1 mg kg^{-1}) and methadone (0.5 mg kg^{-1})]; XMO [xylazine (0.1 mg kg^{-1}) and morphine (0.5 mg kg^{-1})], and XT [xylazine (0.1 mg kg^{-1}) and tramadol (5 mg kg^{-1})]. Each drug combination was mixed in the syringe and injected intravenously. Sedation, heart rate (HR), mean arterial blood pressure (MAP), rectal temperature (RT °C), respiratory rate (f_R), arterial blood gases and electrolytes were measured before drug administration (T0) and then at 15 minute intervals for 120 minutes (T15–T120).

Results Heart rate significantly decreased in all treatments compared with T0. $PaCO_2$ values in XM and XMO were higher at all time points compared with T0. In treatments X and XM, pH, bicarbonate (HCO_3^-) and base excess were increased at all time points compared with T0. PaO_2 was significantly decreased at T15–T75 in XM, at all time points in XMO, and at T15 and T30 in XT. Sedation at T15 and T30 in XM and XMO was greater than in the other treatments.

Conclusions and clinical relevance The combinations of methadone, morphine or tramadol with xylazine resulted in cardiopulmonary changes similar to those induced by xylazine alone in sheep. The combinations provided better sedation, principally at 15 minutes and 30 minutes following administration.

Keywords α_2-agonists, opioids, ovine, sedation.

Introduction

Xylazine is 10–20 times more potent in ruminants than in other species (Kästner 2006). Cardiopulmonary effects include bradycardia, changes in arterial blood pressure, tachypnea

accompanied by pulmonary edema and arterial hypoxemia (Bacon et al. 1998; Kästner 2006). Death resulting from the development of pulmonary edema after administration of xylazine has also been reported (Uggla & Lindqvist 1983). Despite these profound cardiopulmonary effects, sedation may not be as pronounced as expected, and recumbency may not be induced when xylazine is administered to sheep intravenously (IV) or intramuscularly (IM) (Kästner 2006). Therefore, combining xylazine with other drugs may be useful to enhance sedation.

Morphine and methadone are classified as full μ-agonists and are widely used in veterinary practice, alone or in combination with other drugs for premedication and analgesia. However, the reported use of these drugs in ruminants is rare. Studies on drug residues are lacking, which may limit the clinical usefulness of opioids in food animal practice (KuKanich & Papich 2009).

Tramadol may be classified as an atypical opioid drug because much of its analgesic action is attributable to a central effect in inhibiting the reuptake of serotonin and noradrenaline, and it also shows relatively weak action at opioid μ-receptors (KuKanich & Papich 2009). Adverse effects following its administration include sedation, although there are few data on its administration as part of a premedication or sedation protocol in sheep. Guedes et al. (2005) demonstrated that tramadol alone administered IM to dogs prior to general anesthesia did not produce any visible sedation. Excitatory effects on the central nervous system (CNS), such as agitation and nystagmus, following the administration of opioids to ruminants have been described (Waterman et al. 1990, 1991; Lin & Riddell 2003; Edmondson et al. 2012), and may counteract the level of sedation observed.

Combinations of sedatives and opioid drugs are commonly used in veterinary anesthesia because they have useful synergistic effects. This synergism enhances sedation and analgesia and may facilitate a significant reduction in the doses of both drugs, thereby reducing the adverse

cardiopulmonary effects associated with each drug when it is administered alone. Numerous studies in dogs and cats have demonstrated that sedation is better when an α_2-agonist is administered in combination with an opioid than when the α_2-agonist is administered alone (Selmi et al. 2003; Leppänen et al. 2006; Monteiro et al. 2008; Cardoso et al. 2014).

The aim of this study was to examine the cardiopulmonary and sedative effects of xylazine in combination with different opioids when administered IV in sheep. The study hypothesis was that sedation would be superior following the administration of these combinations compared with the administration of xylazine alone.

Materials and methods

This research was conducted with the approval and supervision of the Ethics Committee on Animal Use of the University of Franca, Brazil (protocol no. 038/12). All procedures were conducted in compliance with the ethical principles of good practice in animal experimentation.

Animals

Six female, non-pregnant Santa Inês sheep, with a mean ± standard deviation (SD) age of 12 ± 8 months and mean ± SD weight of 39.5 ± 7.4 kg were used. The animals were kept collectively in 6 × 6 m plots and were given hay, pelleted feed and mineral supplements on a daily basis, and water *ad libitum*. Prior to the study, the health of the animals was evaluated using a complete blood count, liver and renal biochemical profile, and fecal parasitologic examination. For at least 20 days prior to the initiation of the study, the animals were monitored for individual behavior and were conditioned to physical restraint. Before the study, food and water were withheld for 24 hours and the hair over the right jugular vein and auricular arteries was clipped.

Once the animals were moved to the experimental area, the skin sites for vessel catheterization were aseptically prepared. A catheter (18 gauge, 2.5 cm Safelet; Nipro Medical Ltda, SP, Brazil) was introduced into the right jugular vein, and a second catheter (20 gauge, 2.5 cm) introduced into an auricular artery with the sheep restrained in a standing position. The ambient temperature was 22 °C. Fifteen minutes were allowed to elapse following instrumentation before any measurements were recorded.

Experimental design

The sheep were randomized (by drawing of lots) to four treatments in a crossover study, with a minimum interval of 7 days between treatments. The four treatments were: X, xylazine (0.1 mg kg^{-1}; Rompun 2%; Bayer AG, SP, Brazil); XM, xylazine (0.1 mg kg^{-1}) and methadone (0.5 mg kg^{-1}; Mytadon, 10 mg mL^{-1}; Cristália Produtos Químicos e Farmacêuticos Ltda, SP, Brazil); XMO, xylazine (0.1 mg kg^{-1}) and morphine (0.5 mg kg^{-1}; Dimorf, 10 mg mL^{-1}; Cristália Produtos Químicos e Farmacêuticos Ltda), and XT, xylazine (0.1 mg kg^{-1}) and tramadol (5 mg kg^{-1}; Tramadon, 50 mg mL^{-1}; Cristália Produtos Químicos e Farmacêuticos Ltda). Each drug combination was mixed in the syringe. The final volume was adjusted to 5 mL with 0.9% sodium chloride to facilitate blinding and administered IV into the jugular catheter over 30 seconds. The catheter was then flushed with 0.9% sodium chloride. Animals were then left to wander freely in the experiment area. Data for all variables were collected by the same investigator for all animals at all evaluation times. Variables were measured before drug administration (baseline, T0) and every 15 minutes after drug administration for 120 minutes (T15–T120).

Degree of sedation

Three evaluators who were unaware of the treatment assessed the degree of sedation. Sedation was scored using a numerical rating scale of 0–10, on which a score of 0 indicates no sedation and a score of 10 indicates recumbency with no movement (Kästner et al. 2003) (Appendix 1). Assessments of sedation were always performed prior to the measurement of other variables and evaluators' scores were averaged.

Cardiopulmonary variables

Cardiopulmonary data were collected in the following order. Heart rate (HR) was measured using transthoracic auscultation with a stethoscope in the region of the fourth left intercostal space for 1 minute. Mean arterial blood pressure (MAP) was evaluated using the arterial catheter connected to a system filled with 0.1% heparin solution (50 IU mL^{-1}). Pressure was measured intermittently using an aneroid manometer (Indústria Bic de Aparelhos Médicos Ltda, SP, Brazil) which was calibrated against a mercury column before use. Prior to measurement, the system was zeroed using the air–saline junction at the point of the shoulder in standing and sternally recumbent animals and the xiphoid process in laterally recumbent animals as reference points. Respiratory rate (f_R) was assessed by observing the movements of the thorax for 1 minute. Rectal temperature (RT °C) was measured with a clinical mercury-in-glass thermometer (Thermometer BD; Becton Dickinson Indústrias Cirurgicas SA, MG, Brazil) inserted into the rectum. Arterial blood samples were taken from the arterial catheter for determination of pH, arterial partial pressure of carbon dioxide ($PaCO_2$), arterial partial pressure of oxygen (PaO_2), base excess (BE), arterial hemoglobin oxygen saturation (SaO_2), sodium (Na^+), potassium (K^+), ionized calcium (iCa^{2+}) and chloride (Cl^-) concentrations. Each blood sample amounted to 0.5 mL and was withdrawn into a disposable syringe containing heparin and

sealed with a rubber stopper. Samples were analyzed immediately using a blood gas analyzer [Cobas b 121; Roche Diagnostics (Schweiz) AG, Switzerland].

Statistical analysis

Data were analyzed using GraphPad Prism Version 5.0 (GraphPad Software, Inc., CA, USA). Data were tested for normality prior to analysis using the Shapiro–Wilk test. Normally distributed data were analyzed using analysis of variance (ANOVA) for repeated measures; Dunnett's test was used to compare data within the same treatment group. ANOVAs and a *post hoc* Tukey test were used to compare data between treatments. Non-parametric data were analyzed using the Kruskal–Wallis test with a *post hoc* Dunn's test. For all analyses, a p-value of < 0.05 was considered to indicate statistical significance.

Results

All animals were included in the study and all completed the 120 minute evaluation period, recovering without complications. Behavioral effects other than sedation included drooling, bruxism, vocalization and facial or generalized tremors (Table 1).

Sedative effects

Sedation scores were significantly higher compared with baseline at T15–T105 in X ($p < 0.01$) and at T15–T90 in XM ($p < 0.001$) and at all time points in XMO ($p < 0.01$) and XT ($p < 0.001$) (Fig. 1). In XM, sedation was significantly greater at T15 in comparison with T90, T105 and T120 ($p < 0.01$). In XMO, sedation scores were significantly greater at T15, T30 and T60 in comparison with T105 and T120. In XT, sedation at T30 was significantly greater than at T120. When sedation scores were compared between treatments, those at T15 and T30 were significantly higher in XM and XMO than in X and XT. At T30, the sedation score in XT was significantly higher than in X ($p < 0.05$).

Sternal or lateral recumbency (scores 6–10) occurred in variable numbers of animals in XM at three time points (T15, T30, T45), in XMO at six time points (T15–T90) and in XT at three time points (T15, T60, T90). Recumbency was not observed in any animal administered xylazine alone.

Cardiopulmonary variables and body temperature

Heart rate decreased in all treatments at all time points in comparison with baseline ($p < 0.001$) (Table 2). There was no significant difference in HR among treatments. MAP was significantly decreased from baseline in X at T45 and T90 ($p < 0.05$) and in XMO at T15 ($p < 0.0001$), T45 and T75 ($p < 0.001$) and T60 ($p < 0.05$). In XT, MAP was significantly higher at T15 and T75 than in the other treatments.

Rectal temperatures were unchanged, except at T60 in X ($p < 0.05$), and did not differ among treatments.

In comparison with baseline values, f_R was significantly lower at T60–T120 in X ($p < 0.05$) and T45–T90 in XMO ($p < 0.001$) (Table 2). There were no significant differences among treatments.

Blood gas and electrolyte variables

Significant increases in pH compared with baseline were measured at all time points in X and XM, and at T45–T120 in XMO ($p < 0.001$) (Table 3). In a comparison among treatments, pH values in X and XM were significantly higher at T45 and T120. $PaCO_2$ values were higher at all time points than at baseline ($p < 0.001$) in XM and XMO. In the comparative analysis among the treatments, no significant difference was observed. Analysis of BE revealed a significant increase (values became more positive) at all time points compared with T0 in X and XM

($p < 0.0001$), and at T30–T120 in XMO and XT ($p < 0.05$). There were no statistically significant differences among treatments.

In comparison with baseline values, PaO_2 was significantly lower at T15–T75 in XM ($p < 0.01$), at all time points in XMO ($p < 0.001$), and at T15 and T30 in XT ($p < 0.001$). In comparison with values in treatment X, PaO_2 values were significantly lower at T30–T60 in XM ($p < 0.01$), T15 in XMO ($p < 0.01$) and T30 in XT ($p < 0.05$).

There were no statistically significant differences in values of SaO_2, Na^+, K^+, iCa^{2+} and Cl^- in either comparisons with baseline values or among treatments, and values remained within the physiologic ranges for this species.

Discussion

The combination of a sedative (such as an α_2-agonist) with an opioid is used very commonly in veterinary anesthetic practice. Recommended doses of xylazine for IV administration to sheep vary markedly. Grant & Upton (2001) found that 0.05 mg kg^{-1} of xylazine IM resulted in poor sedation, whereas administration of 0.1 mg kg^{-1} xylazine IM resulted in obvious signs of sedation, but the animals remained standing (Shokry et al. 1976). Marked sedative effects were observed at doses of > 0.3 mg kg^{-1} IV (Hsu et al. 1987, 1989). A dose of 0.1 mg kg^{-1} xylazine was chosen for this study in order to achieve sedation sufficient for comparison with the effects of xylazine–opioid combinations.

Equipotent doses of morphine, methadone and tramadol for sheep are not reported in the literature. Consequently, the dose rates used in this study were based on those in studies that compared the use of morphine with tramadol or methadone in dogs (Mastrocinque & Fantoni 2003; Maiante et al. 2009).

202 The time course of 120 minutes chosen for this study was based on what is known about the
203 pharmacokinetic characteristics of xylazine in sheep (Garcia-Villar et al. 1981) and the sedative
204 effects of morphine, methadone and tramadol in combination with dexmedetomidine in dogs
205 (Cardoso et al. 2014).
206 Adverse side effects such as tremors, bruxism, nystagmus and vocalization were prevalent in all
207 the xylazine–opioid treatments. Waterman et al. (1990, 1991) demonstrated that butorphanol and
208 fentanyl administration in sheep leads to agitation and distressed behavior. Lin & Riddell (2003)
209 observed slow horizontal nystagmus following IV administration of detomidine and butorphanol
210 in dairy cattle. In the same species, sedation after the administration of xylazine and butorphanol
211 was no greater than that after xylazine alone (Levine et al. 1992). Alpacas exhibited neurologic
212 signs of hyperexcitability, hyperesthesia, tremors and ataxia after administration of tramadol IV
213 (Edmondson et al. 2012). The adverse effects observed in the present study were similar, further
214 supporting the excitatory effects of opioids on the CNS in ruminant species.
215 Following the IV administration of α_2-agonists, there is a biphasic blood pressure response.
216 Initially, hypertension caused by peripheral vasoconstriction occurs, after which a reflex
217 bradycardia is mediated by baroreceptors. In humans, a prolonged, centrally mediated
218 hypotensive period then ensues, but hypotension after the administration of α_2-agonists has not
219 been reported in dogs (Murrell & Hellebrekers 2005). In sheep, the administration of xylazine
220 (0.05 mg kg^{-1} IM or 0.2 mg kg^{-1} IV) did not induce significant changes in HR (Bacon et al.
221 1998; Grant & Upton 2001). Opioids can cause cardiovascular depression by inducing negative
222 chronotropy and decreased cardiac output (Stanley et al. 1980). However, in goats, 0.6 mg kg^{-1}
223 methadone IV did not cause alterations in HR for up to 240 minutes (Olsén et al. 2013).
224 Similarly, in dogs, the administration of morphine at 3 mg kg^{-1} IV did not induce significant

changes in HR during 30 minutes of evaluation (Priano & Vatner 1981). IV tramadol at doses of 1.5 mg kg^{-1} and 2.6 mg kg^{-1} followed by a continuous infusion caused no change in HR in dogs over 45 minutes (Seddighi et al. 2009). The effects of combinations of an α_2-agonist with an opioid on MAP have not been described in sheep. In dogs administered xylazine and methadone, systolic arterial blood pressure fell but hypotension did not occur over the 60 minute study period (Monteiro et al. 2008). In the present study, although MAP was significantly decreased at some time points, hypotension (MAP < 60 mmHg) was not observed. HR decreased during all treatments, but at no time was clinically significant bradycardia noted.

The administration of α_2-agonists may result in a decrease in body temperature caused by depression of the thermoregulatory centre (Pypendop & Verstegen 1998) and reduced muscular activity (Virtanen 1989). In the present study, the significant decrease in the RT of sheep identified at one time point after xylazine administration was not clinically significant, and no significant decreases in RT occurred in the other treatments. Administration of methadone (0.5 mg kg^{-1} and 1 mg kg^{-1} IV) and morphine (1 mg kg^{-1} IV) to dogs resulted in a progressive reduction of body temperature for up to 90 minutes (Maiante et al. 2009). Monteiro et al. (2009) reported that body temperature decreased in dogs administered tramadol 2 mg kg^{-1} IV and acepromazine, although the vasodilatory effects of acepromazine are likely to be responsible for this change.

Dexmedetomidine and medetomidine administered to sheep do not change f_R (Kästner et al. 2001). $PaCO_2$ was not significantly changed after IV administration of 0.15 mg kg^{-1} and 0.2 mg kg^{-1} xylazine to sheep (Celly et al. 1997; Bacon et al. 1998), despite the occurrence of apnea for 30 seconds followed by tachypnea for up to 60 minutes (Celly et al. 1997). Opioids may cause respiratory depression by decreasing the ventilatory response to hypercapnia (Steffey

et al. 1993); however, hypercapnia did not occur after the administration of morphine or methadone (0.5 mg kg^{-1}) IV to conscious dogs (Maiante et al. 2009). There is evidence that tramadol has little effect on ventilation in some species when administered at doses lower than those used in this study (Mastrocinque & Fantoni 2003). In the present study, f_R decreased significantly at 60–120 minutes in X and 45–120 minutes in XMO, and mild but statistically significant increases in $PaCO_2$ were observed in the XM and XMO treatments for the duration of monitoring. Sedation with treatment XM resulted initially in lateral recumbency and with XMO in sternal recumbency, which may have limited lung expansion and resulted in decreased ventilation.

Determination of pH can be used as an indicator of homeostasis (Sobiech et al. 2005). The pH and BE values in all treatments showed a progressive increase with time. Elevations of pH, bicarbonate (HCO_3^-) and BE have been reported following epidural xylazine in sheep (Aminkov & Hubenov 1995) and IV xylazine in goats (Mogoa et al. 2000), although none of these authors suggested why these changes occurred. Ringer et al. (2013) analyzed the acid-base and electrolyte effects of a continuous 3 hour infusion of romifidine or xylazine in horses and noted increasing pH, HCO_3^- and BE. The authors hypothesized that this change was attributable to hypochloremia resulting from urinary loss. No significant electrolyte abnormalities were identified in the present study and therefore the mechanism by which such changes occur in sheep remains uncertain.

Hypoxemia [$PaO_2 < 60$ mmHg (7.9 kPa)] in sheep after administration of an α_2-agonist is common, especially when the drug is administered IV (Kästner 2006). The proposed mechanism of action is intense venous spasm mediated via adrenoreceptor agonism, intense pulmonary congestion, increased microvascular pressure and alveolar capillary rupture, resulting in an

inflammatory response (Celly et al. 1997; Bacon et al. 1998; Kästner et al. 2007). As previously discussed, opioids may cause respiratory depression (Steffey et al. 1993), leading to hypoxemia, particularly when the animal is breathing atmospheric air. However, in dogs, the administration of methadone or morphine at the same doses used in this study did not induce changes in PaO_2 (Maiante et al. 2009). Likewise, tramadol 2 mg kg^{-1} did not cause alterations in PaO_2 in dogs (Mastrocinque & Fantoni 2003). In this study, PaO_2 was decreased, particularly when combinations of xylazine and an opioid were administered, and these changes were statistically significant. This decrease may reflect the level of sedation as sheep administered a xylazine–opioid combination were more likely to become recumbent, which may have adverse effects on ventilation perfusion matching. Mitchell & Williams (1976) measured arterial oxygen concentrations in healthy, conscious sheep and found them to be significantly lower in laterally recumbent than in standing animals. However, in the present study, at no point did the decrease in PaO_2 translate to a clinically significant decrease in SaO_2. It must be emphasized that oxygen should be provided to sedated sheep if possible, particularly when the animal is at greater risk for hypoxemia from lateral or dorsal positioning, is unfasted or has systemic disease.

In conclusion, opioids used in combination with xylazine in sheep potentiate the sedative effect of xylazine in a similar way to that described in other species; methadone and morphine appear to promote better sedation. As the number of animals included in this study was small, and the doses of drugs were restricted, further work is necessary to ascertain the most suitable combination for clinical use. The combinations depressed HR in a manner similar to that induced by xylazine alone, but MAP was maintained. Although PaO_2 was reduced in all treatments, SaO_2 values did not indicate the need for oxygen therapy.

Acknowledgements

The authors would like to thank Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP: 2013/00831-0) for the provision of financial support, and Ana Márcia Zago and the Design Department of the University of Franca for drafting the figure.

References

Aminkov BY, Hubenov HD (1995) The effect of xylazine epidural anaesthesia on blood gas and acid-base parameters in rams. Br Vet J 151, 579–585.

Bacon PJ, Jones JG, Taylor P et al. (1998) Impairment of gas exchange due to alveolar oedema during xylazine sedation in sheep; absence of a free radical mediated inflammatory mechanism. Res Vet Sci 65, 71–75.

Cardoso CG, Marques DR, da Silva TH et al. (2014) Cardiorespiratory, sedative and antinociceptive effects of dexmedetomidine alone or in combination with methadone, morphine or tramadol in dogs. Vet Anaesth Analg 41, 636–643.

Celly CS, McDonell WN, Young SS et al. (1997) The comparative hypoxaemic effect of four α_2-adrenoceptor agonists (xylazine, romifidine, detomidine and medetomidine) in sheep. J Vet Pharmacol Ther 20, 464–471.

Edmondson MA, Duran SH, Boothe DM et al. (2012) Pharmacokinetics of tramadol and its major metabolites in alpacas following intravenous and oral administration. J Vet Pharmacol Ther 35, 389–396.

Garcia-Villar R, Toutain PL, Alvinerie M et al. (1981) The pharmacokinetics of xylazine hydrochloride: an interspecific study. J Vet Pharmacol Ther 4, 87–92.

Grant C, Upton RN (2001) Cardiovascular and haemodynamic effects of intramuscular doses of xylazine in conscious sheep. Aust Vet J 79, 58–60.

317 Guedes AGP, Natalini CC, Rude EP et al. (2005) Comparison of tramadol and morphine for pre-
318 medication of dogs undergoing general anaesthesia for orthopedic surgery. [Abstract.] Vet
319 Anaesth Analg 32, 236.
320 Hsu WH, Schaffer DD, Hanson CE (1987) Effects of tolazoline and yohimbine on xylazine-
321 induced central nervous system depression, bradycardia, and tachypnea in sheep. J Am Vet Med
322 Assoc 190, 423–426.
323 Hsu WH, Hanson CE, Hembrough FB et al. (1989) Effects of idazoxan, tolazoline, and
324 yohimbine on xylazine-induced respiratory changes and central nervous system depression in
325 ewes. Am J Vet Res 50, 1570–1573.
326 Kästner SBR (2006) α_2-agonists in sheep: a review. Vet Anaesth Analg 33, 79–96.
327 Kästner SBR, Boller M, Kutter A et al. (2001) Clinical comparison of preanaesthetic
328 intramuscular medetomidine and dexmedetomidine in domestic sheep. Dtsch Tierärztl
329 Wochenschr 108, 409–413.
330 Kästner SBR, Wapf P, Feige K et al. (2003) Pharmacokinetics and sedative effects of
331 intramuscular medetomidine in domestic sheep. J Vet Pharmacol Ther 26, 271–276.
332 Kästner SBR, Ohlerth S, Pospischil A et al. (2007) Dexmedetomidine-induced pulmonary
333 alterations in sheep. Res Vet Sc 83, 217–226.
334 KuKanich B, Papich MG (2009) Opioid analgesic drugs. In: Veterinary Pharmacology and
335 Therapeutics (9th edn). Riviere JE, Papich MG (eds). Wiley-Blackwell, USA. pp. 301–336.
336 Leppänen MK, McKusick BC, Granholm MM et al. (2006) Clinical efficacy and safety of
337 dexmedetomidine and buprenorphine, butorphanol or diazepam for canine hip radiography. J
338 Small Anim Pract 47, 663–669.

339 Levine HD, Dodman NH, Hustead D (1992) Evaluation of a xylazine–butorphanol combination
340 for use during standing laparotomy in dairy cattle. Agri Pract 13, 19–23.
341 Lin HC, Riddell MG (2003) Preliminary study of the effects of xylazine or detomidine with or
342 without butorphanol for standing sedation in dairy cattle. Vet Ther 4, 285–291.
343 Maiante AA, Teixeira Neto FJ, Beier SL et al. (2009) Comparison of the cardio-respiratory
344 effects of methadone and morphine in conscious dogs. J Vet Pharmacol Ther 32, 317–328.
345 Mastrocinque S, Fantoni DT (2003) A comparison of preoperative tramadol and morphine for
346 the control of early postoperative pain in canine ovariohysterectomy. Vet Anaesth Analg 30,
347 220–228.
348 Mitchell B, Williams JT (1976) Respiratory function changes in sheep associated with lying in
349 lateral recumbency and with sedation by xylazine. Vet Anaesth Analg 6, 30–36.
350 Mogoa EG, Stegmann GF, Guthrie AJ (2000) Effects of xylazine on acid-base balance and
351 arterial blood-gas tensions in goats under different environmental temperature and humidity
352 conditions. J S Afr Vet Assoc 71, 229–231.
353 Monteiro ER, Figueroa CDN, Choma JC et al. (2008) Effects of methadone, alone or in
354 combination with acepromazine or xylazine, on sedation and physiologic values in dogs. Vet
355 Anaesth Analg 35, 519–527.
356 Monteiro ER, Junior AR, Assis HM et al. (2009) Comparative study on the sedative effects of
357 morphine, methadone, butorphanol or tramadol, in combination with acepromazine, in dogs. Vet
358 Anaesth Analg 36, 25–33.
359 Murrell JC, Hellebrekers LJ (2005) Medetomidine and dexmedetomidine: a review of
360 cardiovascular effects and antinociceptive properties in the dog. Vet Anaesth Analg 32, 117–127.

Olsén L, Olsson K, Hydbring-Sandberg E et al. (2013) Methadone in healthy goats – pharmacokinetics, behaviour and blood pressure. Res Vet Sci 95, 231–237.

Priano LL, Vatner SF (1981) Morphine effects on cardiac output and regional blood flow distribution in conscious dogs. Anesthesiology 55, 236–243.

Pypendop BH, Verstegen JP (1998) Hemodynamic effects of medetomidine in the dog: a dose titration study. Vet Surg 27, 612–622.

Ringer SK, Schwarzwald CC, Portier K et al. (2013) Blood glucose, acid-base and electrolyte changes during loading doses of α_2-adrenergic agonists followed by constant rate infusions in horses. Vet J 198, 684–689.

Seddighi MR, Egger CM, Rohrbach BW et al. (2009) Effects of tramadol on the minimum alveolar concentration of sevoflurane in dogs. Vet Anaesth Analg 36, 334–340.

Selmi AL, Mendes GM, Lins BT et al. (2003) Evaluation of the sedative and cardiorespiratory effects of dexmedetomidine, dexmedetomidine–butorphanol, and dexmedetomidine–ketamine in cats. J Am Vet Med Assoc 222, 37–41.

Shokry M, Morad HM, Khalil IA (1976) Studies on the effect of Rompun in sheep. Vet Med Rev 2, 237–243.

Sobiech O, Lew M, Lew S et al. (2005) The effect of propofol on acid-base balance and ionic composition of venous and arterial blood in goats. Pol J Vet Sci 8, 295–300.

Stanley TH, Liu WS, Webster LR et al. (1980) Haemodynamic effects of intravenous methadone anaesthesia in dogs. Can Anaesth Soc J 27, 52–57.

Steffey EP, Eisele JH, Baggot JD et al. (1993) Influence of inhaled anesthetics on the pharmacokinetics and pharmacodynamics of morphine. Anesth Analg 77, 346–351.

Uggla A, Lindqvist A (1983) Acute pulmonary oedema as an adverse reaction to the use of xylazine in sheep. Vet Rec 113, 42.

Virtanen R (1989) Pharmacological profiles of medetomidine and its antagonist, atipamezole. Acta Vet Scand 85 (Suppl), 29–37.

Waterman AE, Livingston A, Amin A (1990) The antinociceptive activity and respiratory effects of fentanyl in sheep. J Assoc Vet Anaesth 17, 20–23.

Waterman AE, Livingston A, Amin A (1991) Analgesic activity and respiratory effects of butorphanol in sheep. Res Vet Sci 51, 19–23.

Appendix 1 Numerical rating scale for assessment of sedation in sheep (Kästner et al. 2003)

Score	Behavior
0	Standing, alert, normal behavior
1	Standing, alert, reduced head and ear movements
2	Standing, slight head drop
3	Standing, moderate head drop
4	Standing, severe head drop and ataxia
5	Standing, severe head drop and severe ataxia
6	Sternal recumbency, head up
7	Sternal recumbency, unable to support head
8	Lateral recumbency, occasional attempts to attain sternal recumbency
9	Lateral recumbency, uncoordinated head and leg movements
10	Lateral recumbency, no movements

Figure 1 Sedation scores in six sheep after intravenous administration of (a) xylazine (0.1 mg kg^{-1}) (X), (b) xylazine (0.1 mg kg^{-1}) and methadone (0.5 mg kg^{-1}) (XM), (c) xylazine (0.1 mg kg^{-1}) and morphine (0.5 mg kg^{-1}) (XMO) or (d) or xylazine (0.1 mg kg^{-1}) and tramadol (5 mg kg^{-1}) (XT). Sedation scores of ≥ 6 indicate sternal recumbency and scores of ≥ 8 indicate lateral recumbency. Lines indicate median scores, boxes indicate interquartile ranges and whiskers indicate ranges. *Significantly different from baseline within the same treatment (X and XMO: $p < 0.01$; XM and XT: $p < 0.001$). †Significantly different from X and XT at the same time point ($p < 0.05$). ‡Significantly different from X at the same time point ($p < 0.05$).

403 **Table 1** Adverse effects observed in six sheep over 120 minutes in four treatment conditions:

404 intravenous (IV) xylazine (0.1 mg kg^{-1}) (treatment X); IV xylazine (0.1 mg kg^{-1}) and methadone

405 (0.5 mg kg^{-1}) (treatment XM); IV xylazine (0.1 mg kg^{-1}) and morphine (0.5 mg kg^{-1}) (treatment

406 XMO), and IV xylazine (0.1 mg kg^{-1}) and tramadol (5 mg kg^{-1}) (treatment XT)

407 NB, normal behavior.

408

Treatment	**Time points (minutes)**						
	T15	**T30**	**T45**	**T60**	**T75**	**T90**	**T1**
X	Drooling	Drooling	Drooling	Drooling Urination	Drooling Urination	Drooling Urination	NI
XM	Drooling Bruxism Mydriasis Nystagmus Facial tremors	Drooling Bruxism Mydriasis Generalized tremors	Drooling Bruxism	Drooling Bruxism Vocalization	Drooling Vocalization	Vocalization	NI
XMO	Drooling Bruxism Facial tremors	Drooling Bruxism	Vocalization Bruxism Mydriasis Drooling	Bruxism Mydriasis Vocalization Drooling	Vocalization Drooling	Vocalization Bruxism	Vo
XT	Bruxism Vocalization Drooling Facial tremors	Drooling Bruxism Increased response to touch	Drooling Urination	Drooling Urination Vocalization	Vocalization	NB	NI

Table 2 Mean ± standard deviation heart rate (HR), mean arterial pressure (MAP), rectal temperature (RT °C) and respiratory rate (f_R) in sheep before (T0) and after intravenous administration of xylazine (X), xylazine and methadone (XM), xylazine and morphine (XMO) or xylazine and tramadol (XT)

	Treatment	Time points (minutes)							
		T0	**T15**	**T30**	**T45**	**T60**	**T75**	**T90**	**T105**
	X	101 ± 14	85 ± 11*	73 ± 14*	71 ± 17*	70 ± 16*	74 ± 14*	75 ± 16*	77 ± 9*
e^{-1})	XM	124 ± 14	70 ± 11*	77 ± 14*	78 ± 15*	86 ± 16*	83 ± 14*	88 ± 9*	92 ± 9*
	XMO	112 ± 12	77 ± 15*	78 ± 13*	73 ± 7*	71 ± 9*	72 ± 7*	79 ± 9*	90 ± 14*
	XT	105 ± 13	70 ± 9*	71 ± 9*	69 ± 12*	71 ± 12*	77 ± 15*	76 ± 16*	84 ± 19
	X	110 ± 11	99 ± 11	99 ± 7	97 ± 14*	98 ± 8	103 ± 9	95 ± 12*	99 ± 10
	XM	103 ± 10	93 ± 9	92 ± 8	94 ± 14	99 ± 12	97 ± 9	99 ± 7	99 ± 12
	XMO	111 ± 14	89 ± 11*	99 ± 8	94 ± 12*	97 ± 13*	93 ± 12*	104 ± 6	104 ± 10
	XT	109 ± 8	107 ± 9†	106 ± 6	110 ± 15	114 ± 12	115 ± 12†	106 ± 17	111 ± 13
	X	39.1 ± 0.5	39.2 ± 0.4	39.0 ± 0.4	38.9 ± 0.5	38.8 ± 0.6	38.9 ± 0.5	38.9 ± 0.6	39.1 ± 0.7
	XM	39.3 ± 0.4	39.1 ± 0.5	38.9 ± 0.6	38.9 ± 0.7	39.0 ± 0.8	39.2 ± 0.8	39.3 ± 0.7	39.5 ± 0.7
	XMO	39.7 ± 0.6	39.6 ± 0.7	39.4 ± 0.6	39.2 ± 0.6	39.2 ± 0.5	39.3 ± 0.4	39.4 ± 0.4	39.4 ± 0.4
	XT	39.3 ± 0.3	39.4 ± 0.8	39.1 ± 0.6	39.0 ± 0.6	39.0 ± 0.7	38.8 ± 0.7	38.9 ± 1.0	39.2 ± 0.8
	X	28 ± 7	27 ± 11	25 ± 10	24 ± 11	21 ± 6*	20 ± 5*	21 ± 6*	19 ± 7*
ute^{-1})	XM	23 ± 10	21 ± 5	18 ± 4	23 ± 7	19 ± 5	18 ± 4	19 ± 4	19 ± 2
	XMO	28 ± 6	24 ± 5	24 ± 8	20 ± 4*	21 ± 2*	19 ± 4*	21 ± 3*	22 ± 8
	XT	26 ± 4	29 ± 9	25 ± 12	21 ± 7	21 ± 8	24 ± 10	20 ± 7	20 ± 4

*Significantly different from T0 within the same treatment ($p < 0.05$). †Significantly different from other treatments at the same time point ($p < 0.05$).

Table 3 Mean ± standard deviation arterial pH (pH), arterial partial pressure of carbon dioxide ($PaCO_2$), arterial partial pressure of oxygen (PaO_2), bicarbonate (HCO_3^-) and base excess (BE) in sheep sedated with xylazine (X), xylazine and methadone (XM), xylazine and morphine (XMO) or xylazine and tramadol (XT)

atment	Time points (minutes)							
	T0	**T15**	**T30**	**T45**	**T60**	**T75**	**T90**	**T105**
	7.46 ± 0.02	7.50 ± 0.02*	7.52 ± 0.01*	7.53 ± 0.02*†	7.52 ± 0.04*†	7.54 ± 0.03*†	7.52 ± 0.02*†	7.53 ± 0.0
	7.45 ± 0.06	7.48 ± 0.03*	7.50 ± 0.03*	7.51 ± 0.02*†	7.52 ± 0.02*†	7.53 ± 0.03*†	7.52 ± 0.04*†	7.53 ± 0.0
O	7.41 ± 0.08	7.44 ± 0.06	7.46 ± 0.04	7.49 ± 0.03*	7.50 ± 0.03*	7.52 ± 0.02*	7.51 ± 0.02*	7.51 ± 0.0
	7.47 ± 0.07	7.48 ± 0.06	7.50 ± 0.04	7.51 ± 0.03	7.51 ± 0.04	7.52 ± 0.03	7.51 ± 0.04	7.51 ± 0.0
	32 ± 3	33 ± 3	34 ± 2	35 ± 3	33 ± 3	32 ± 2	34 ± 3	33 ± 3
	29 ± 3	35 ± 1*	36 ± 3*	37 ± 1*	36 ± 3*	34 ± 2*	34 ± 1*	34 ± 3*
O	28 ± 3	32 ± 2*	33 ± 3*	33 ± 2*	33 ± 3*	32 ± 3*	34 ± 3*	33 ± 2*
	31 ± 3	34 ± 4	34 ± 4	34 ± 3	35 ± 3	34 ± 2	35 ± 4	32 ± 5
	4.2 ± 0.4	4.3 ± 0.4	4.5 ± 0.2	4.6 ± 0.4	4.3 ± 0.4	4.2 ± 0.2	4.5 ± 0.4	4.3 ± 0.4
	3.8 ± 0.4	4.6 ± 0.1*	4.7 ± 0.4*	4.9 ± 4.9*	4.7 ± 0.4*	4.5 ± 0.2*	4.5 ± 0.1*	4.5 ± 0.4*
O	3.7 ± 0.4	4.2 ± 0.2*	4.3 ± 0.4*	4.3 ± 0.2*	4.3 ± 0.4*	4.2 ± 0.4*	4.5 ± 0.4*	4.3 ± 0.2*
	4.1 ± 0.4	4.5 ± 0.5	4.5 ± 0.5	4.5 ± 0.4	4.6 ± 0.4	4.5 ± 0.2	4.6 ± 0.5	4.3 ± 0.6
	80 ± 3	71 ± 10	73 ± 6	75 ± 5	77 ± 10	75 ± 9	77 ± 3	73 ± 3
	81 ± 4	65 ± 8*†	65 ± 8*†	65 ± 8*†	69 ± 6*	71 ± 7*	76 ± 6	75 ± 5
O	84 ± 3	63 ± 6*†	70 ± 5*	73 ± 7*	67 ± 5*	73 ± 3*	73 ± 5*	75 ± 8*
	81 ± 1	64 ± 10*	64 ± 6*†	74 ± 5	75 ± 4	76 ± 5	74 ± 5	73 ± 6
	10.6 ± 0.4	9.4 ± 1.3	9.7 ± 0.7	9.9 ± 0.6	10.2 ± 1.3	9.9 ± 1.1	10.2 ± 0.3	9.7 ± 0.3

	10.7 ± 0.5	8.6 ± 1.0*†	8.6 ± 1.0*†	8.6 ± 1.0*†	9.1 ± 0.7*	9.4 ± 0.9*	10.1 ± 0.7	9.9 ± 0.6
O	11.1 ± 0.4	8.3 ± 0.7*†	9.3 ± 0.6*	9.7 ± 0.9*	8.9 ± 0.6*	9.7 ± 0.3*	9.7 ± 0.6*	9.9 ± 1.0*
	10.6 ± 0.1	8.5 ± 1.3*	8.5 ± 0.7*†	9.8 ± 0.6*	9.9 ± 0.5	10.1 ± 0.6	9.8 ± 0.6	9.7 ± 0.7
	21 ± 2	25 ± 2*	27 ± 3*	28 ± 2*	28 ± 4*	27 ± 3*	27 ± 3*	27 ± 3*
	20 ± 3	26 ± 2*	27 ± 2*	29 ± 1*	29 ± 2*	28 ± 2*	27 ± 2*	27 ± 2*
O	23 ± 2	22 ± 4	23 ± 4	25 ± 3	25 ± 2	26 ± 2	26 ± 3*	26 ± 1
	22 ± 5	25 ± 4*	26 ± 4*	27 ± 3*	27 ± 4*	27 ± 3*	27 ± 4*	25 ± 4
	− 2 ± 2	2 ± 1*	4 ± 2*	5 ± 2*	5 ± 4*	5 ± 3*	4 ± 2*	4 ± 2*
	− 3 ± 4	2 ± 2*	4 ± 2*	6 ± 1*	6 ± 2*	5 ± 2*	4 ± 3*	4 ± 2*
O	− 5 ± 4	− 2 ± 5	0 ± 4*	2 ± 3*	2 ± 3*	3 ± 2*	3 ± 2*	3 ± 1*
	− 1 ± 6	2 ± 4	4 ± 4*	4 ± 3*	5 ± 3*	4 ± 3*	4 ± 4*	3 ± 3*

*Statistically different from T0 within the same treatment ($p < 0.05$). †Statistically different from all other treatments at the same time point ($p < 0.05$).