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# The bioavailability of medetomidine in eight sheep following oesophageal administration

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ABSTRACT

There is sound evidence that medetomidine is an effective analgesic for acute pain in sheep. In this study, 15 μg $kg^{-1}$ of medetomidine was administered intravenously, and into the oesophagus, in a cross-over study, using eight sheep. Following intravenous administration, medetomidine could be detected in the plasma of these sheep for 120–180 min but following oesophageal administration, medetomidine could not be detected in the plasma of any sheep at any of 17 time points over four days. It is suspected that this is due to high first pass metabolism in the liver. Consequently, we conclude that future studies investigating the use of analgesics in orally-administered osmotic pumps in sheep should consider higher doses of medetomidine (e.g. >100 μg $kg^{-1}$), further investigations into the barriers of medetomidine bioavailability from the sheep gut, liver-bypass drug delivery systems, or other $\alpha_2$-adrenergic agonists (e.g. clonidine or xylazine).



## 1. Introduction

Medetomidine is an $\alpha_2$-selective adrenergic agonist that has been used in veterinary medicine as a sedative, analgesic and skeletal muscle relaxant (Posner and Burns, 2009). It is registered only for use in dogs (and cats in some countries) but it has also been investigated in horses (England and Clarke, 1996), donkeys (Lizarraga and Janovyak, 2013), cattle (Ranheim et al., 1999), pigs (Sakaguchi et al., 1992), sheep (Kästner, 2006) and a wide range of non-domestic species (Jalanka, 1989).

In sheep, medetomidine has been shown to be an effective analgesic, especially for acute pain (Lizarraga and Chambers, 2012). In 1992, Chambers demonstrated that 5 μg $kg^{-1}$ IV of medetomidine provided a similar level of analgesia to 15 μg $kg^{-1}$ IV of fentanyl in sheep, as assessed by nociceptive threshold testing using mechanical stimulation. These sheep were apparently healthy and were not exposed to any noxious stimuli (besides the mechanical stimulation itself) at any time during this experiment. Later, Muge et al. (1994) showed that 2–7 μg $kg^{-1}$ IV of medetomidine raised the nociceptive threshold of similarly non-painful sheep in a dose-dependent manner. Depending on dose, this analgesic effect was detected for up to 60 min. In a more recent study, Murdoch et al. (2013) used osmotic pumps to demonstrate that an intraperitoneal infusion of medetomidine in sheep at 3 μg $kg^{-1}$ $h^{-1}$ significantly decreased pain scores for 10 h. Moreover, this analgesia was detected independent of sedation.

The studies by Muge et al. (1994) and Murdoch et al. (2013) show the inextricable association between the method and route of drug administration, and its duration of effect. There are a number of commercially-available orally-administered drugs for ruminants that utilise various drug delivery mechanisms to provide sustained effects. As two examples, Ivomec Maximizer® Controlled Release Capsules (Merial) and Rumensin Capsules (Elanco) are able to deliver ivermectin and monensin, respectively, into the reticulorumen for 100 days using plastic-bodied "winged" capsules with tableted cores. Methods of drug administration like these offer attractive options for the sustained-administration of analgesic drugs (e.g. $\alpha_2$-agonists) to sheep.

There have been a number of studies that have reported on the pharmacokinetics and pharmacodynamics of $\alpha_2$-agonists following administration into the alimentary tract in animals but the majority of these have focussed on the buccal transmucosal route of administration (Malone and Clarke, 1993; Sleeman et al., 1997; Ansah et al., 1998; Freeman and England, 1999; Ramsay et al., 2002; Slingsby et al., 2009; Gardner et al., 2010; Naples et al., 2010; DiMaio Knych and Stanley, 2011; Kaukinen et al., 2011; I'Ami et al., 2013; Hopfensperger et al., 2013). Furthermore, a commercially-available preparation of buccal transmucosal detomidine gel suitable for horses is manufactured by

*Abbreviations:* $C_{max}$, maximum plasma concentration; EDTA, ethylenediaminetetraacetic acid; HPLC, high performance liquid chromatography; IU, international units.

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Orion Pharma (Orion Corporation, Finland) and is distributed by Elanco (Domosedan Gel®) in Europe and Zoetis (Dormosedan Gel®) in USA and Australia.

In contrast to these reports on the *buccal transmucosal* absorption of $\alpha_2$-agonists, other publications exist that have investigated the *gastrointestinal* absorption of four $\alpha_2$-agonists: dexmedetomidine (Proctor et al., 1991; Anttila et al., 2003), detomidine (Devitt, 1989), medetomidine (Vainio, 1988) and clonidine (Davies et al., 1977; Larsson et al., 2011). We were unable to find any studies that report on the gastrointestinal bioavailability of medetomidine in sheep but the limited data available suggests that the bioavailability of $\alpha_2$-agonists from the gastrointestinal system varies depending on species, $\alpha_2$-agonist and the methodology of the study.

Combining all of these findings, the intriguing question can be raised as to whether an analgesic that is effective in sheep, such as medetomidine, can be delivered to sheep in a way that provides a sustained effect and is easy to administer, and additionally, is without a prohibitive suite of adverse side effects, such as excessive sedation. Our theory was that osmotic pumps containing medetomidine, administered orally to sheep, might be able to achieve these aims. Consequently, the aim of this preliminary investigation was to quantify the bioavailability of medetomidine in sheep following administration into the oesophagus.

## 2. Materials and methods

Approval of our experimental protocol was provided by the Murdoch University Animal Ethics Committee which adheres to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

Eight adult merino sheep (seven ewes and one wether) of similar weight were randomly selected and purchased from a sales yard for inclusion in this study. Their ages were unknown but based on their dentition were estimated to be 2–6 years of age. Sheep were transported to Murdoch University and placed onto pasture for one week. During the study, the sheep were held in individual raised pens (1.5 × 1 m). The pens were adjacent to each other and allowed eye contact between animals. Oaten hay and water were provided ad libitum, and each sheep was also given approximately one kilogram of lupins at the end of each day.

A catheter (16 G, 83 mm, Becton Dickinson Angiocath, North Ryde, Australia) was placed into a jugular vein (secured with tissue glue) of each sheep at the commencement of the study. Extension sets (75 cm, priming volume 1.6 mL, BMDi TUTA Healthcare, Sydney, Australia) were then attached to the catheters and secured to the neck of each animal with an adhesive bandage. Approximately 2 mL of blood was collected from each sheep for baseline analyses. All blood samples were transferred to EDTA blood collection tubes. Collected blood was centrifuged at 2000 ×*g* for 10 min at 4 °C within 1 h of sampling. Plasma was frozen at −80 °C until analysis.

Four of the eight sheep were randomly selected and administered 15 μg $kg^{-1}$ of medetomidine (Sedamed™, Ceva Animal Health, Australia, 1 mg $mL^{-1}$) IV in the opposite jugular vein at zero minutes. At 1, 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 90, 120, 240 and 360 min, approximately 5 mL of blood was collected from the jugular catheter and held in a syringe. A separate syringe was then used to collect an additional 2 mL of blood. The initial aspirate of blood, which was being held in the first syringe, was then injected back into the jugular vein. Withdrawn blood was always auto-transfused within 15 s of collection. The catheter was then flushed with 5 mL of heparinised saline (5 IU $mL^{-1}$).

The remaining four sheep were administered 15 μg $kg^{-1}$ of medetomidine (Zalopine®, Orion Corporation Animal Health, Turku, Finland, 5 mg $mL^{-1}$, diluted to 0.3 mg $mL^{-1}$ with isotonic saline) into an 8 FG 500 mm sterile canine urinary catheter (Henry Schein, New York, USA) which in turn was in the lumen of the tube from a Magrath calf feeder (Springer Magrath, Minnesota, USA) that had been positioned into the oesophagus (see Fig. 1). Drug was administered at zero minutes and urinary catheters were then immediately flushed with 20 mL of isotonic saline before the urinary catheter and Magrath calf feeder tube were removed from each animal's oesophagus. Blood was collected using the same method previously described for the IV group. Blood samples were collected at 0, 30, 60 and 90 min, then at 2, 3, 4, 5, 6, 12 and 24 h, and at 2, 3, 4 and 5 days. On day 8, following a one week washout period, the two groups of four sheep were crossed over and the experiment was repeated. To calculate bioavailability, the area under the plasma drug concentration-time curve (AUC) following oesophageal administration was divided by AUC following intravenous administration.

To confirm the presence of medetomidine in the preparation we used, and to determine if the urinary catheter altered the concentration of medetomidine that passed through it, diluted medetomidine (Zalopine®, diluted to 0.3 mg $mL^{-1}$ using isotonic saline) was flushed through two urinary catheters into two plain glass tubes (0 h). Two more urinary catheters were filled with diluted medetomidine but samples were not collected from these catheters until 24 h later (24 h). The concentrations of medetomidine were then compared to diluted medetomidine that had not been exposed to a urinary catheter (pre-catheter).

Samples were prepared and analysed using a method of medetomidine detection that has been validated for selectivity, recovery, precision, linearity and limits of detection (Netto et al., 2011). Briefly, samples were extracted by solid phase extraction (SPE) using 30 mg Bond Elut Plexa (Varian Inc., Palo Alto, USA) 1 mL cartridges. Standards and samples were injected individually in 1 μL volumes into an Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA, USA). The analytical column used was a Pursuit XRs Ultra diphenyl (Varian Inc.) with dimensions of 30 mm × 2.0 mm × 3 μm. The HPLC was coupled to an Agilent Classic series ion trap mass spectrometer with an electrospray ionization source (Agilent Technologies) in positive ionization mode. Tandem mass spectrometry (MS/MS) of medetomidine was carried out using the transition *m/z* 201 ➔ 95.

The heart rate, respiratory rate, rectal temperature, frequency of rumen contractions, recumbency and demeanour of the sheep were also recorded. Demeanour was assessed subjectively by three veterinarians experienced in large animal medicine (THH, GCM & FRM) as being alert and responsive, quiet and responsive, mildly sedated, moderately sedated or heavily sedated. To investigate the effect that medetomidine had on heart rate and respiratory rate, data were tested for normality using a Shapiro–Wilk test and statistically significant differences between the time = 0 data points and subsequent data points were

**Fig. 1.** Oesophageal administration of medetomidine in a sheep. A Magrath calf feeder was positioned into the oesophagus of the sheep and a 500 mm canine urinary catheter was then advanced through the lumen of the Magrath calf feeder. Drug was then administered into the urinary catheter. The catheter was then flushed with isotonic saline.