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Pharmacokinetics and pharmacodynamics of dexmedetomidine in horses

Sarah E. Shane
seshane7@gmail.com

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By

Sarah E. Shane

Approved by:

Claudio C. Natalini (Major Professor)
Robert E. Meyer
Robin Fontenot
Cory Langston
Todd Archer
Larry Hanson (Graduate Coordinator)
Kent H. Hoblet (Dean, College of Veterinary Medicine)

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Name: Sarah E. Shane

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Major Professor: Claudio C. Natalini

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Candidate for Degree of Master of Science

An ideal dexmedetomidine protocol has yet to be determined for standing sedation in horses. It was hypothesized that an intravenous (IV) bolus followed by continuous rate infusion (CRI) dexmedetomidine would have a quicker increase in plasma concentrations compared to repeated intramuscular (IM) injections. In a cross-over design, eight adult, female horses were randomly placed in two groups: the CRI group (IV bolus dexmedetomidine at 0.005 mg/kg followed by a CRI at 0.01 mg/kg/hr for 15 minutes then 0.005 mg/kg/hr for 60 minutes) and the IM group (dexmedetomidine at 0.01 mg/kg, followed by 0.005 mg/kg in 30-minute intervals for 60 minutes). Analgesia was evaluated using a mechanical pressure threshold device. Intravenous dexmedetomidine produced faster onset of sedation and increased pressure threshold compared to IM administration. Individual horses had a large variability in dexmedetomidine plasma concentrations between CRI and IM administration.

DEDICATION

I would like to dedicate this research to my parents, Steven and Robin Shane, for their continuous love and support. They have always been my biggest advocate for achieving my goals and providing me with guidance throughout my professional pursuits.

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CHAPTER I

LITERATURE REVIEW

Background

Equine anesthesia has been refined over time to include a multi-modal approach to anesthesia and analgesia. Currently, protocols include inhalation anesthesia, total intravenous anesthesia (TIVA), partial intravenous anesthesia (PIVA), and standing sedation. Common anesthetic drugs used in equine anesthesia include inhalant anesthetics (e.g. isoflurane and sevoflurane), phenothiazines (eg. acepromazine), dissociative anesthetics (eg. ketamine), benzodiazepines (eg. diazepam and midazolam), opioids (eg. butorphanol, morphine, and hydromorphone), and alpha-2 adrenergic agonists (eg. xylazine, detomidine, romifidine, and dexmedetomidine). Among domestic species undergoing general anesthesia, equines have the highest complication rate (Vigani & Garcia-Pereira, 2014).

Canine and feline anesthetic mortalities are reported to be 0.17% and 0.24%, respectively (Brodbelt et al., 2008). With sedation, mortalities are reported to be 0.07% and 0.12%, respectively (Brodbelt et al., 2008). Cardiovascular and respiratory causes were the two predominant causes of mortality in dogs and cats (Brodbelt et al., 2008). This mortality rate is lower compared to equine. A retrospective study evaluating approximately 40,000 cases of equine anesthetic events, both emergency and non-emergency, concluded that the overall mortality rate was 1.9%; at 0.9% for non-emergency cases and 11.7% for emergency cases (e.g.

colics and abdominal surgeries) (Johnston et al., 2002). Common intraoperative complications include hypotension, respiratory complications (ie. hypercapnia and hypoxemia), cardiac arrhythmias, and hemorrhage (Wagner, 2008) (Johnston et al., 2002). Post-anesthetic complications include fractures, myopathies, and peripheral neuropathies. It has been reported that in non-emergency cases, 0.23% of horses die from fractures and 0.07% die from myopathies (Johnston et al., 2002). Factors that increase anesthetic risk are increased duration of anesthesia, increased age, and surgery outside of normal working hours (Johnston et al., 2002; Vigani & Garcia-Pereira, 2014).

Intra- and post-operative complications can be minimized when general anesthesia is replaced by standing sedation; however, standing sedation has its list of complications. Common complications involved with standing sedation include excessive sedation, muscle relaxation, ataxia, and hypersensitivity (Vigani & Garcia-Pereira, 2014). These complications can be reduced when using a continuous rate infusion (CRI) that can be titrated to effect. A CRI allows for more consistent sedation compared to bolus administration (Vigani & Garcia-Pereira, 2014). Further research is needed to determine if dexmedetomidine, a common drug used in equine medicine, can be administered as a bolus dose followed by a CRI to allow for standing sedation.

Dexmedetomidine: An Overview

Alpha-2 Adrenergic Receptor Agonists

Alpha-2 (α2) receptors are found throughout the body in the peripheral and central nervous system, platelets, liver, and kidney (Gertler et al., 2001). Alpha-2 adrenergic agonists initiate their effect by activating the G-coupled protein receptors (Gertler et al., 2001) (Grimm et al., 2015). Once the G proteins are activated, a second messenger system is activated. When

activated, the second messenger system will inhibit adenylate cyclase which will decrease the formation of 3,5-cyclic adenosine monophosphate (cAMP) (Gertler et al., 2001). Alpha-adrenergic receptors are present on the pre- and post-synaptic junctions. Alpha-2 agonists exhibit a negative feedback loop at the pre-synaptic junction where the decrease in cAMP will inhibit the release of norepinephrine causing vasodilation and a decrease in gastrointestinal motility (Giovannitti et al., 2015) (Valverde, 2013) (Gertler et al., 2001). Post-synaptic activation of the alpha-2 receptors will cause vasoconstriction and inhibit insulin release (Valverde, 2013) (Gertler et al., 2001).

Adrenergic receptors have been classified into α and β receptors that have different physiologic outcomes when stimulated. Four subtypes of α2 receptors have been discovered and each one of these has different effects when stimulated. Alpha-2_A and $\alpha2_C$ receptors are located within the central nervous system and $\alpha2_B$ receptors are located within the vascular smooth muscle (Giovannitti et al., 2015). The locus coeruleus, located in the pons, expresses a high density of $\alpha2_A$ receptors (Wang et al., 1996). Due to this expression, it has been concluded that the locus coeruleus is responsible for the sedative and hypnotic effects of alpha2 adrenergic receptor agonists (Gertler et al., 2001).

Dexmedetomidine is an alpha-2 adrenergic receptor agonist often used in equine medicine for its sedative and analgesic properties. Dexmedetomidine is the dextrorotary isomer of medetomidine (Giovannitti et al., 2015) with a higher selectivity for α2 adrenoreceptors (α2:α1 ratio of 1620:1) (Gertler et al., 2001). Dexmedetomidine has an advantage over drugs (eg. xylazine and detomidine) that have a higher selectivity for α1 receptors as they are more likely to cause

rigidity and paradoxical excitement (Grimm et al., 2015). The receptor subtype for alpha-1 is the Gq type while the receptor subtype for alpha-2 is the Gi type (Taylor & Cassagnol, 2020). Alpha-1 receptors are primarily found within the vascular smooth muscle and bronchi. Agonists will cause smooth muscle contraction (Piascik & Perez, 2001) that will lead to an increase in systemic vascular resistance causing hypertension (Valverde, 2010).

Alpha-2 adrenergic agonists have a variable effect on the cardiovascular system due to the stimulation of both alpha-1 and alpha-2 receptors. Upon initial administration, alpha-2 adrenergic agonists will cause an increase in systemic vascular resistance leading to a decrease in heart rate and cardiac output (Bloor et al., 1992). An increase in dexmedetomidine plasma concentration has led to a decrease in cardiac output, causing a 12% decrease in dexmedetomidine clearance (Dutta et al., 2000). Systemic vascular resistance will return to normal with remaining bradycardia due to a decrease in central sympathetic outflow (Grimm et al., 2015).

Pharmacodynamics of Dexmedetomidine

Alpha-2 adrenergic agonists are commonly associated with cardiovascular depression due to an increase in systemic vascular resistance leading to a decrease in heart rate and cardiac output. A 5 µg/kg bolus of dexmedetomidine, when given to a standing horse, has been shown to significantly decrease the heart rate up to 10 minutes following bolus administration with heart rate returning to baseline at 20 minutes (Rezende et al., 2015). A bolus of 3.5 µg/kg of dexmedetomidine in standing horses will decrease the stroke volume at 5 minutes with a decrease in the cardiac index at 5 and 10 minutes after bolus administration (Bettschart-Wolfensberger et al., 2005). A 3.5 µg/kg bolus of dexmedetomidine increases the blood pressure

at 5 minutes, causes a decrease at 20, 30, and 45 minutes and returns to normal at 60 minutes (Bettschart-Wolfensberger et al., 2005). This change of blood pressure is commonly seen following the administration of alpha-2 adrenergic agonists as previously described. Horses receiving PIVA with isoflurane and a dexmedetomidine CRI show a greater reduction in heart rate when receiving a 1.75 µg/kg/hr CRI compared to 0.5 µg/kg/hr CRI (Bettembourg et al., 2019). The study by Bettembourg, et al. (2019) did not include an initial bolus dose of dexmedetomidine, and the dexmedetomidine plasma concentrations were decreased in the 0.5 µg/kg/hr CRI group compared to the 1.75 µg/kg/hr CRI group which could account for the difference in heart rate. When an initial bolus dose of 8 µg/kg of dexmedetomidine is administered followed by a 1.75 µg/kg/hr CRI, there is a 21% decrease in cardiac index with a 57% increase in total peripheral resistance and a 16% decrease in heart rate (Risberg, 2016).

Dexmedetomidine has been shown to affect respiratory parameters. The respiratory rate decreased following IV administration of 3.5 ug/kg of dexmedetomidine in ponies and a 5 ug/kg IV bolus of dexmedetomidine in horses (Bettschart-Wolfensberger et al., 2005; Rezende et al., 2015). When administered as a CRI at of 1.75 µg/kg/hr in conjunction with isoflurane, dexmedetomidine will decrease respiratory drive, as evidenced by an increase in P_aCO_2 and a decrease in P_aO_2 (Marcilla et al., 2012). However, when an IV dexmedetomidine CRI of 1.75 µg/kg/hr is used for standing sedation, blood gas values to remain within normal limits even with a significant reduction in respiratory rate (Bettschart-Wolfensberger et al., 2005).

Gastrointestinal motility decreases following alpha-2 adrenergic agonist administration. Alpha-2 adrenergic agonists exhibit a dose-dependent inhibition on spontaneous and electrically-induced

motility in the small intestine due to the presence of alpha-2 adrenoceptors (Zullian et al., 2011). Both IV and IM administration of 30 μg/kg detomidine will decrease gastrointestinal motility with an earlier onset and greater magnitude when given IV (Mama et al., 2009). A 5 μg/kg bolus of dexmedetomidine will result in a decrease of borborygmi for 60 minutes after administration (Rezende et al., 2015).

Analgesia for Nociceptive Equine Pain

Pain Pathway

Nociceptors are a group of receptors responsible for nociception; the detection of painful stimuli followed by a withdrawal reflex (Sneddon, 2018). There are two types of nociceptors. The first receptor is the Aδ fibers. These receptors are myelinated, have a medium diameter, and respond quickly to acute stimulation (Basbaum et al., 2009). The Aδ fibers can be further differentiated into type I and type II classes. The type I class responds to mechanical and chemical stimuli while the type II class responds to mechanical stimuli (Basbaum et al., 2009). The second type of receptor is the C fibers. These receptors are unmyelinated, small in diameter, and slow in their response (Basbaum et al., 2009).

Pain can be further divided into acute and chronic pain. Acute pain is considered a brief, noxious stimulus that is generated through the nociceptive pathways (Le Bars et al., 2001). Chronic pain is consistent with injury or disease that alters the normal function of peripheral nerves causing alterations within the pain pathway (Basbaum et al., 2009). This pain is physiological, psychological, and can inhibit recovery (Basbaum et al., 2009).

Evaluation of Equine Nociception

Evaluation of equine analgesia has been evaluated through mechanical, thermal, and electrical nociceptive threshold testing (NTT) (Love et al., 2011). A drug is considered to have anti-nociceptive properties when there is an increase in the nociceptive threshold when the drug is administered (Gozalo-Marcilla et al., 2020). Behavioral reactions are deemed the most reliable indicators of pain sensation compared to biochemical indicators (Le Bars et al., 2001). Behavioral reactions vary based on where the stimulus is being applied. These reactions can include escape, avoidance, or aggression as well as modification of behavior (Le Bars et al., 2001). Biochemical indicators include catecholamine and corticoid levels and opioid requirements (Le Bars et al., 2001). Nociceptive threshold testing can be used both clinically and experimentally. Clinically it is used to evaluate the response to treatment. Experimentally, nociceptive threshold testing has been used to evaluate the efficacy of an analgesic protocol and to determine the necessary threshold end-points (Love et al., 2011).

Electrical threshold testing involves the application of an electrical stimulus. Stimulation will activate nerves based on their diameter. The first nerves to be activated are Aβ then Aδ followed by C fibers (Le Bars et al., 2001). The advantages of electrical stimuli are that it is quantifiable, reproducible, and noninvasive (Le Bars et al., 2001). A disadvantage of electrical threshold testing is that it is not a natural stimulus experienced in an animal's environment (Le Bars et al., 2001). Additionally, there is no consensus as to current and electrode location, and this method is less reliable when compared to mechanical and thermal threshold testing (Luna et al., 2015).

Thermal threshold testing will stimulate superficial pain through cutaneous receptors (Le Bars et al., 2001). Thermal stimulation can be tested through two different approaches. These approaches are (1) evaluating the delay of response to a constant temperature and (2) measuring the temperature when a response occurs to a rapid increase in temperature (Love et al., 2011). One type of thermal threshold testing involves the use of radiant heat. This method involves focusing heat on a clipped area of the horse and evaluates the length of time before a behavioral reaction occurs (Love et al., 2011). A more common type of thermal threshold testing in horses uses a thermal probe. The thermal probe contains a heating element as well as a temperature sensor that is applied over a clipped area of skin (Love et al., 2011).

The mechanical nociceptive threshold (MNT) is evaluated by using a pressure algometer. Mechanonociceptive (Aδ) and nociceptive (C) fibers are responsible for sensing the mechanical stimuli (Basbaum et al., 2009). One type of pressure algometer includes a 1 cm^2 rubber plunger tip with a pressure range of 1-20 kg/cm^2 (Haussler & Erb, 2006). This pressure algometer has previously been validated in equine mechanical NTT (Haussler & Erb, 2006; Haussler et al., 2007; Mama et al., 2009) A positive response to MNT includes the horse pawing the ground, shifting weight away from the stimulus, or turning around and looking at the stimulus. Disadvantages of this type of nociceptive testing include sensitization or injury to local tissue (Le Bars et al., 2001) and inconsistency in operator application of consistent pressure (Love et al., 2011).

Current Analgesia Protocols

Equine analgesic protocols include locoregional anesthetics (e.g. lidocaine and bupivacaine) and systemic analgesics (e.g. alpha-2 adrenergic agonists, opioids, and sodium channel blockers).

The addition of these drugs enhances analgesia, contributes to sedation, and reduces minimum alveolar concentration (MAC). Common alpha-2 adrenergic agonists include xylazine, detomidine, romifidine, and dexmedetomidine.

When xylazine is administered as a 1 mg/kg IV bolus, the onset of analgesia is within 5 minutes and lasts for 40 minutes as determined by electrical stimulation (Seo et al., 2011). A bolus dose of IV detomidine (30 µg/kg) provides maximum nociceptive threshold at 30 minutes when compared to an IM bolus dose that occurs later than 30 minutes and with a decrease in magnitude (Mama et al., 2009). A dexmedetomidine CRI of 1.75 µg/kg/hr in combination with isoflurane does not lower the nociceptive threshold when electrical stimulation is applied to the lateral palmar digital nerve (Risberg, 2016). The authors attributed this lack of response to electrical stimulation to additional drugs that were given during the induction period. In standing sedation, when 5 µg/kg of dexmedetomidine is administered IV, MNT will be increased for 30 minutes following the bolus and return to baseline at 45 minutes (Rezende et al., 2015). Dexmedetomidine given IV at 2, 4, and 6 µg/kg/hr are considered anti-nociceptive with plasma levels between 0.02 – 0.647 ng/ml (Risberg et al., 2014). A dexmedetomidine CRI of 2 µg/kg/hr IV does not show significant anti-nociceptive effect from electrical stimulation, suggesting that a CRI of 4 µg/kg/hr or greater may be needed for analgesia to accomplish dexmedetomidine plasma levels >0.15 ng/mL (Risberg et al., 2014).

Opioids are commonly added to equine anesthesia for their analgesic properties as well as to decrease the amount of additional anesthetic and inhalant drugs required (Bennett & Steffey, 2002). Common opioids used in horses are butorphanol, morphine, and hydromorphone.

Morphine given at 0.66 mg/kg IM provides analgesia for superficial pain lasting approximately 30 minutes while butorphanol at 0.22 mg/kg IM provides visceral pain relief for up to 4 hours (Kalpravidh et al., 1984). In horses, hydromorphone doses at 0.04 mg/kg and 0.08 mg/kg IV result in an increase in thermal nociceptive threshold from 15 minutes after administration up to 12 hours after treatment (Reed et al., 2019).

Lidocaine, an amide sodium channel blocker, has been used systemically in horses for multiple purposes including reducing pain, inflammation, and inhalant requirements, and improving gastrointestinal motility (Doherty & Seddighi, 2010; Malone et al., 2006; Rezende et al., 2011). Given as an IV CRI, lidocaine has a sevoflurane MAC reducing effect ranging from 6.3 to 44.6% (Rezende et al., 2011). An IV lidocaine CRI between 40 µg/kg/min and 60 µg/kg/min results in plasma levels between 644 to 1790 ng/ml and shows an increase in nociceptive threshold to single and repeated stimulation (Risberg et al., 2014).

Dexmedetomidine Continuous Rate Infusion with Inhalant Anesthesia

PIVA is commonly utilized in equine anesthesia to decrease inhaled agent MAC and reduce respiratory and cardiovascular side effects. Commonly used volatile anesthetics in equine anesthesia include isoflurane and sevoflurane. Isoflurane is used more frequently due to its greater potency, low blood solubility, and lower cost compared to sevoflurane (Sleiman et al., 2016). When combined with an IV dexmedetomidine CRI of 1.75 µg/kg/hr, isoflurane has a MAC reduction of 15.6% (Sleiman et al., 2016). A further MAC reduction of 53 ± 15 % can be achieved by administering a 3.5 µg/kg bolus prior to initiating the CRI (Gozalo-Marcilla et al., 2013). Dexmedetomidine improves recovery scores following general anesthesia (Gozalo-Marcilla et al., 2013; Risberg, 2016). A 0.875 µg/kg IV bolus of dexmedetomidine prior to

recovery provides uneventful recoveries with fewer attempts to stand and longer times in lateral recumbency (Gozalo-Marcilla et al., 2013; Marcilla et al., 2012). A dexmedetomidine CRI intraoperatively (1.75 µg/kg/hr IV) results in superior recovery scores compared to saline infusions (Risberg, 2016). This study showed a recovery visual analog score (VAS) of 10 ± 5 mm in the dexmedetomidine group while the saline group had a recovery VAS of 46 ± 23 mm. The recovery visual analog score was graded from 0 -100 mm with 0 mm representing the best recovery and 100 mm representing the worse recovery (Risberg, 2016).

Equine Standing Sedation

Equine standing sedation has been beneficial in equine medicine to assist in standing surgical procedures. Anesthetic protocols may include IV xylazine, detomidine, or dexmedetomidine CRIs. Head height (HH) has been used as an evaluation of sedation for various anesthetic protocols and allows for a quantitative measurement (Ringer et al., 2013; Schauvliege et al., 2019). Detomidine given as a bolus dose (30 µg/kg) IV decreases HH within 10 minutes, with a more gradual decrease noted when the bolus is given IM (Mama et al., 2009). Dexmedetomidine IV (5 µg/kg) shows a reduction in HH for 4-60 minutes following administration with a mean reduction of 70% with HH following bolus administration returning to baseline at 60 minutes (Rezende, et al., 2015). Dexmedetomidine given as a CRI (8 µg/kg/hr IV) reduces HH but returns to normal 65 minutes following CRI discontinuation (Ranheim et al., 2015). Sedation evaluated using a VAS increases with increasing dexmedetomidine CRI doses (Risberg et al., 2014). On sedation, a VAS of 0 mm represents no sedation with 100 mm representing heavy sedation (Risberg, et al., 2014). Auditory stimulation is decreased following both detomidine and dexmedetomidine boluses (Mama et al., 2009; Rezende et al., 2015).

Pharmacokinetics of Dexmedetomidine

The compartmental analysis evaluates the distribution of drugs to central (e.g. plasma and heart) and peripheral (e.g. fat) compartments within the body. Noncompartmental (moment) analysis is utilized to calculate pharmacokinetic parameters without evaluation of distribution throughout compartments (Dyck et al., 1993).

Dexmedetomidine is a highly protein-bound drug and metabolism is through biotransformation by glucuronidation and cytochrome P450 in the liver (Gertler et al., 2001; Weerink et al., 2017). Approximately 95% of the metabolites are excreted through the urine with 4% excreted through the feces (Gertler et al., 2001). Dexmedetomidine plasma levels in horses have been evaluated through both non-compartmental (Bettschart-Wolfensberger et al., 2005; Ranheim et al., 2015; Rezende et al., 2015) and 2-compartment models (Grimsrud et al., 2015) (Table 1.1).

Table 1.1 Comparative Equine Dexmedetomidine Research

Author	Administration	Route	C_{max} (ng/ml)	V_{ss} (ml/kg)	Cl (ml/kg/min)	$T_{1/2}$ (min)
Ranheim, et al., 2014	150 min CRI: 8 µg/kg/hr	IV	0.63 ± 0.44	13600 ± 7900	440 ± 270	20.3 ± 5.8
Rezende, et al., 2014	5 µg/kg	IV	5.7 ± 3.52	1053. 52 ± 472.58	78.62 ± 59.97	8.03 ± 0.84
Bettschart-Wolfensberger, et al., 2005 (Mature Ponies)	3.5 µg/kg	IV	3.77 ± 1.52			28.96 ± 7.61
Bettschart-Wolfensberger, et al., 2005 (Young Ponies)	3.5 µg/kg	IV	4.6 ± 2.86			19.8 ± 9.63

Comparison of dexmedetomidine standing sedation protocols and associated pharmacokinetics expressed as mean ± SD. C_{max}: maximum drug plasma concentration; V_{ss}: volume of distribution at steady state (ml kg^{-1}), Cl: clearance (ml kg^{-1} hr^{-1}); $T_{1/2}$: elimination half-life.

Dexmedetomidine concentration exhibits large variations among conscious horses (Ranheim et al., 2015; Rezende et al., 2015) compared to concentrations while under general anesthesia (Risberg, 2016). Following a 3.5 µg/kg IV bolus of dexmedetomidine in awake ponies, plasma concentrations decrease rapidly and fall below the limit of quantification (LOQ) within 2 hours (Bettschart-Wolfensberger et al., 2005). This elimination half-life was prolonged compared to a dexmedetomidine bolus of 5 µg/kg IV that reached the LOQ between 30 and 60 minutes after administration (Rezende et al., 2015).

Dexmedetomidine plasma analysis in other species has included compartmental and non-compartmental analysis (Dent et al., 2019; Dyck et al., 1993; Pypendop et al., 2017). In humans receiving a bolus dose of 2 µg/kg IV, non-compartmental analysis is utilized to determine clearance (0.511 ± 0.125 L/min), elimination half-life (385 ± 144 min), and volume of distribution at steady state (194 ± 28.7 L) (Dyck et al., 1993). A non-compartmental analysis is utilized when evaluating dexmedetomidine plasma levels in dogs (Dent et al., 2019; Vlerick et al., 2020). Using noncompartmental analysis, clearance (8 ± 1.6 ml/kg/min), the volume of distribution at steady state (371 ± 72 ml/kg), and half-life (36 ± 6 minutes) have been determined following a bolus of 5 µg/kg IV. Dexmedetomidine plasma concentrations in cats have been evaluated using a compartmental approach with a one-compartment model being the best fit (Pypendop et al., 2017). A bolus of 25 µg/kg IM when combined with MK-467 has a C_{max} of 801.4 ng/ml and a Cl/F of 5.5 mL/kg/min (Pypendop et al., 2017).

CHAPTER II

PHARMACOKINETICS AND PHARMACODYNAMICS OF INTRAVENOUS CONTINUOUS RATE INFUSION AND REPEATED INTRAMUSCULAR ADMINISTRATION OF DEXMEDETOMIDINE IN STANDING HORSES

Introduction

Alpha-2 (α2) adrenergic receptor agonists are frequently administered both intramuscularly (IM) and intravenously (IV) in equine practice for sedation and as an adjunct to general anesthesia (Muir & Hubbell, 2009). Common drugs include xylazine, detomidine, romifidine, and dexmedetomidine. When a xylazine continuous rate infusion (CRI) is used in combination with butorphanol in horses, constant sedation is produced (Ringer et al., 2012). Medetomidine given as a bolus followed by a CRI gives steady sedation with a decrease in head height (Bettschart-Wolfensberger et al., 1999). Detomidine will also cause a decrease in head height 10 minutes following an IV bolus (Mama, et al., 2009). Side effects commonly seen in relation to αlpha-2 adrenergic receptor agonist administration are related to pre- and post-synaptic activation. Pre-synaptic activation will inhibit norepinephrine release producing vasodilation, and decrease gastrointestinal motility, while post-synaptic activation will cause vasoconstriction and inhibit insulin release (Gertler et al., 2001; Valverde, 2013). The combination of pre-and post-synaptic activation will produce sedation and analgesia (Gertler et al., 2001).

Alpha-2 adrenergic receptor agonists have been previously studied in relation to their analgesic effects. Detomidine administration results in a dose-dependent increase in colorectal and duodenal distention thresholds for nociception (Elfenbein et al., 2009). Sedation, analgesia and plasma concentrations of dexmedetomidine following a single bolus injection (5 μg/kg) have previously been described (Rezende, et al., 2014). Dexmedetomidine was given as a 5 μg/kg bolus resulting in an increase in the mechanical nociceptive threshold for up to 30 minutes with a return to baseline value at 45 minutes after administration (Rezende, et al., 2014). Response to noxious stimuli was evaluated using a previously validated technique with response considered positive if the horse raised its limb, turned towards the stimulus, or shifted away from the pressure (Mama, et al., 2009 and Haussler & Erb, 2006, as cited in Rezende, et al., 2014).
A decrease in gastrointestinal (GI) motility in horses following alpha-2 adrenergic receptor agonist administration is well documented (Grimsrud et al., 2012; Mama et al., 2009; Zullian et al., 2011). In vitro xylazine, medetomidine, and detomidine decreased spontaneous and electrically induced jejunum contractions in a concentration-dependent manner (Zullian, et al., 2011). A decrease in borborygmi has been documented when medetomidine and dexmedetomidine are administered as an IV bolus (Grimsrud, et al., 2012) (Rezende, et al., 2014). Both IV and IM administration of detomidine had similar results with a decrease in GI motility (Mama, et al., 2009).

The pharmacokinetics of dexmedetomidine has been evaluated as a single IV bolus as well as a CRI (Rezende, et al., 2014) (Ranheim, et al., 2014). A large individual variation was shown when evaluating clearance and volume of distribution at a steady state (Rezende, et al., 2014)

(Ranheim, et al., 2014). To the authors' knowledge, at this time, there are no studies evaluating the pharmacokinetics or analgesic scores with dexmedetomidine given as a CRI or IM in standing horses.

The hypothesis was that an IV bolus followed by CRI dexmedetomidine would have a quicker increase in plasma concentrations compared to repeated IM injections leading to a dexmedetomidine plasma concentration difference of 10 ng/ml between the treatment groups. It was anticipated that the difference in dexmedetomidine plasma concentrations would have an effect on selected pharmacodynamic parameters. The hypothesis was evaluated through two separate objectives. The first objective of the study was to describe and compare the pharmacodynamics including heart rate (HR), blood pressure (BP), head height (HH), mechanical pressure threshold (MPT), and GI motility. The second objective of the study was to evaluate plasma concentrations in horses administered dexmedetomidine.

Materials & Methods

The study was approved by the Mississippi State University Institutional Animal Care and Use Committee (Protocol #18-296). Eight, adult, healthy female horses with a mean body weight of 532.63 ± 35.59 kg were enrolled using a blinded, randomized, crossover study design. Prior to the study, each horse had a thorough physical examination and full hematological and clinical chemistry screening. Blood was collected using an 18 gauge, 1-inch needle from the left jugular vein. Horses were given one night to acclimate to their new environment prior to data collection. Horses were kept in box stalls with free choice access to grass hay and water. Neither food nor water were withheld from horses on study days. An experienced anesthetist was blinded to the treatment groups and evaluated HH and MPT.

Horses were randomly assigned to one of the two treatment groups with a three-day washout period between treatments: the CRI group (IV bolus of dexmedetomidine (Orion Corporation, Espoo, Finland) at 0.005 mg/kg followed by a CRI at 0.01 mg/kg/hr for 15 minutes then 0.005 mg/kg/hr for 60 minutes) and the IM group (dexmedetomidine (Orion Corporation, Espoo, Finland) at 0.01 mg/kg followed by 0.005 mg/kg in the same region in 30-minute intervals for 60 minutes). Intramuscular (IM) administration was performed at the cervical area (dorsal to the cervical vertebrae, ventral to the nuchal ligament, and cranial to the spine of the scapula). In each treatment group, saline (0.9% NaCl) was administered at equivalent volumes as the CRI and IM groups to keep the anesthesiologist blinded to the treatment groups. Horses in the IV group would receive saline IM and horses in the IM group would receive saline IV.

On study days, 100 - 200 mg of xylazine (Bimeda-MTC Animal Health Inc., Cambridge, ON Canada) was administered IV to allow for IV catheter placement at least 30 minutes prior to data collection. Horses had one jugular catheter placed in each external jugular vein. The sites were aseptically prepared and short term, 14-gauge, 5.25 inch jugular catheters (Mila International Inc., Florence, KY) were placed. The left jugular catheter was used for drug administration and the right jugular catheter for blood sampling. To determine the pharmacokinetic profile of dexmedetomidine, baseline samples of blood (10 mL) were collected immediately prior to IV and IM administration of dexmedetomidine. Blood samples were collected in an EDTA tube at 0, 5, 10, 20, 30, 45, 60, 90, 120, 150 and 180 minutes throughout the study. After sample collection, blood was placed in an ice bath for no longer than 30 minutes prior to sample

processing. Samples were centrifuged, plasma was harvested and stored at -80 ◦C prior to analysis of dexmedetomidine concentration.

Physiological parameters of heart rate (HR), respiratory rate (RR), systolic blood pressure (SAP), diastolic blood pressure (DAP), and mean arterial blood pressure (MAP), blood glucose (BG), and gastrointestinal motility were assessed before dexmedetomidine administration (baseline) and at 5, 10, 20, 30, 45, 60, 90, 120, 150 and 180 minutes. HR, RR and GI motility were all evaluated based on auscultation. Auscultation for GI motility was performed using a stethoscope and listening to the four abdominal quadrants (right and left paralumbar fossa and caudoventral abdomen) for the duration of 1 minute (Vanderbroek, et al., 2019). Motility was based on a score of 0-3 from a scoring system that was previously published (Sasaki, et al., 2008). A score of 0 represented absent borborygmi. A score of 1 represented a longer period without borborygmi than the period with borborygmi. A score of 2 represented a longer period with borborygmi than the period without borborygmi. A score of 3 represented continuous borborygmi. Arterial blood pressure measurements were obtained using a noninvasive blood pressure technique (NIBP) (Cardell 9401, Sharn Veterinary Inc., Tampa, FL). The blood pressure cuff (23-33 cm in circumference) was placed on the skin at the coccygeal artery and was not corrected to the heart level. BG measurements were taken before the plasma samples were harvested using a glucometer (ReliOn, Bentonville, AR). Head height (cm) and response to noxious stimuli were assessed at 0, 5, 10, 20, 30, 45, 60, 90, 120, 150 and 180 minutes. A measuring tape was used to determine HH from the ground to the tip of the nose in centimeters. Noxious stimulation was evaluated using a mechanical threshold device (Force Dial FDK 60, Wagner Instruments Greenwich, CT, USA). The threshold device accuracy was verified with a 2500 grams test

weight according to the manufacturer. The threshold device was placed on the spine of the scapula and pressure was administered until an adverse reaction was noted. Adverse reactions included moving away from the pressure, head tossing, tail swishing, or stomping. The blinded anesthesiologist was responsible for collecting HH and mechanical nociceptive threshold measurements. Other pharmacodynamic variables were collected by a non-blinded anesthetist.

Dexmedetomidine Plasma Concentration Determination

Working solutions were prepared by dilution of the dexmedetomidine stock solution (Toronto Research Chemicals, Toronto, Canada) with LC/MS grade methanol (Methanol Otima™ Fisher, Lenexa KS). Plasma calibrators were prepared by dilution of the working dexmedetomidine solutions with drug free equine plasma to concentrations ranging from 0.05 to 40 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality control samples (equine drug free plasma fortified with analyte at two concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

Prior to analysis, 0.4 mL of plasma was diluted with 100 µL of water containing the internal standard antipyrine (Sigma Aldrich, St Louis, MO). The samples were vortexed briefly to mix and 100 µL of 5% ammonium hydroxide in saturated sodium chloride solution was added to each sample prior to the addition of 3 mL of MTBE. Samples were mixed by rotation for 20 minutes at 40 revolutions per minute (rpm) and were subsequently centrifuged at 3300 rpm (2260 g) for 5 minutes at 4 ºC and the top organic layer transferred to a glass tube. Samples were dried under nitrogen and dissolved in 120 µL of 5% ACN in water and the injection volume was 30 µL into the liquid chromatography tandem-mass spectrometry (LC-MS/MS) system.

The concentration of dexmedetomidine was measured in plasma by LC-MS/MS. Quantitative analysis was performed on a TSQ Altis triple quadrupole mass spectrometer coupled with a Vanquish liquid chromatography system (Thermo Scientific, Waltham, MA). The spray voltage was 3500V, the sheath and auxillary gas were 50 and 15 respectively (arbitrary units). Product masses and collision energies of each analyte were optimized by infusing the analytes into the mass spectrometer. Chromatography employed an ACE 3 C18 10cm x 2.1mm 3 µm column (Mac-Mod Analytical, Chadds Ford, PA) and a linear gradient of acetonitrile (ACN) in water with a constant 0.2% formic acid at a flow rate of 0.4 ml/min. The initial ACN concentration was held at 5% for 0.20 minutes, ramped to 90% over 4.7 minutes and held at that concentration for 0.1 min before re-equilibrating for 3 minutes at initial conditions.

Detection and quantification were conducted using Selective Reaction Monitoring (SRM) of initial precursor ion for DM (mass to charge ratio *(m/z)* 201.2) and internal standard AP (*(m/z)* 189.1). The response for the product ions for dexmedetomidine (*m/z* 41.2, 68.2, 95.1) and antipyrine (*m/z* 77.1, 131.1, 147.2) were plotted and peaks at the proper retention time integrated using Quanbrowser software (Thermo Scientific). Quanbrowser software was used to generate calibration curves and quantitate DM in all samples. A weighting factor of 1/X was used for all calibration curves.

Dexmedetomidine Pharmacokinetic Analysis

Attempts were made to fit a compartmental model for the IV data, IM data, or both simultaneously. For a compartmental approach, SAAM II (Simulation Analysis and Modeling) was tried, followed by a mixed-effect model attempt using Monolix. For the latter software, this included the Monolix censoring option to address missing concentrations reported as below the

limit of quantitation (BLOQ). In all cases, poor fittings occurred due to high variability in time-concentration values along with BLOQ data points. These BLOQ points occurred while drug administration was still occurring, and in several subjects, during the terminal phase after the last dose was administered or discontinuation of the CRI had occurred.

Noncompartmental analysis (NCA, Moment) parameters were however calculated using standard equations (Blode et al., 2004) using PKNCA (Buckeridge et al., 2015), with the exception of the calculation of mean infusion time described below. The area under the concentration-time curve was calculated using the linear-up/log-down method. Because NCA is most commonly applied to single-dose data there is a misconception that it can only be used for these designs. In actuality, NCA analysis can be performed on multi-dose designs, including extravascular, CRI, and with a bolus followed by CRI(s). The attainment of steady-state is not needed to derive the NCA parameters (Buckeridge et al., 2015; Denney, 2020; Gillespie, 1991).

The derivation of the MRT for the IV bolus followed by two CRI rates in this study did however first require derivation of the Mean Infusion Time (MIT) (Gillespie, 1991). To do so, one must first, determine the cumulative dose across all dosing intervals:

$$Dose(bolus) = 0.005\frac{mg}{kg} \tag{2.1}$$

$$Dose(CRI: 0.01) = 0.01\frac{mg}{kg\ x\ hr}x\ (0.25hr - 0hr) = 0.0025\frac{mg}{kg} \tag{2.2}$$

$$Dose(CRI: 0.005) = 0.005\frac{mg}{kgxhr}x\ (1.25hr - 0.25hr) = 0.005\frac{mg}{kg} \quad (2.3)$$

$$Dose(cumulative) = Dose(bolus) + Dose(CRI: 0.01) + Dose(CRI: 0.005) = 0.0125\frac{mg}{kg} \quad (2.4)$$

The mean infusion time corrects the MRT for the mean time that a molecule of the compound has been in the body. In this case, it would be the mean of the midpoints of the two dosing times. Applying that, it would be:

$$MIT(bolus) = 0hr\ (MIT\ for\ an\ IV\ bolus\ is\ the\ time\ of\ the\ bolus \quad (2.5)$$

$$MIT(CRI: 0.01) = \frac{0.25 - 0}{2} = 0.125\ hr\ (the\ midpoint\ of\ the\ first\ CRI) \quad (2.6)$$

$$MIT(CRI: 0.005) = \frac{1.25 - 0.25}{2} = 0.75\ hr\ (the\ midpoint\ of\ the\ second\ CRI) \quad (2.7)$$

The MIT for the dose regimen is the dose-weighted mean of the MITs for each part of the cumulative dose.

$$MIT(total) = \frac{MIT_{bolus} \times Dose_{bolus} + MIT_{CRI,0.01} \times Dose_{CRI,0.01} + MIT_{CRI,0.005} \times Dose_{CRI,0.005}}{Dose_{cumulative}} \quad (2.8)$$

Thus,

$$MIT = \frac{\left[\left(0hr\ x \frac{0.005mg}{kg}\right) + \left(0.125hr\ x \frac{0.0025mg}{kghr}\right) + \left(0.75hr\ x \frac{0.005mg}{kghr}\right)\right]}{\frac{0.0125mg}{kg}} \quad (2.9)$$

Therefore, MIT = 0.325 hours

The MRT is then produced as MRT = AUMC/AUC – MIT; this correction was manually applied to the PKNCA-calculated results.

The equation for determination of MRT of the IM portion of the study depends on whether nonlinear or linear pharmacokinetics are observed, and typically, MRT for extravascular dosing is calculated as the observed MRT without correction for the MIT (or mean absorption time as would apply for extravascular administration). In the dog, nonlinear dosing is evident. In the cat and human beings, however, dosing linearity appears to exist. Because the vast majority of drugs follow linear dosing, this was the assumption applied to the IM portion of the study (Ho et al., 2015; Ludden, 1991). As such, all standard NCA equations were applied including:

$$Cl = \frac{Fxdose}{AUC}, Vss = MRT\ x\ Cl, and\ F = (\frac{AUCIM}{DoseIM})/(\frac{AUCIV}{DoseIV}) \quad (2.10)$$

Concentrations BLOQ were handled in the standard method for calculating half-life by removing them from the calculation (Buckeridge et al., 2015). The estimated parameters included were

area under the plasma concentration curve to infinity ($AUC_{0-\infty}$) and to the last concentration above the limit of quantification (AUC_{0-last}), area under the moment curve ($AUMC_{0-\infty}$), elimination rate constant (λz), elimination half-life ($t_{1/2}$), volume of distribution at steady state (V_{ss}), maximum concentration (C_{max}) and the time of maximum concentration (T_{max}). For IV data, observed clearance (Cl), mean infusion time (MIT), and mean residence time ($MRT_{0-\infty}$) were also calculated.

Statistical Methods

A minimum sample size of six experimental units for each treatment group was calculated considering a minimum difference of 10 ng/mL dexmedetomidine plasma level, a power of 0.80 and an α-error of 5% for one-tailed test (Kadam & Bhalerao, 2010). The effect of treatment group and time on BG, HR, RR, SAP, DAP, MAP, HP, and MPT were assessed with separate linear mixed models using PROC MIXED in SAS for Windows v9.4 (SAS Institute, Inc, Cary, NC). Group, time point, and their interaction were included as fixed effects. In addition, the period and sequence the horses received the treatments were also included as fixed effects. Horse identity was included as the subject in a random statement. The repeated measures of horse within period were accounted for in a repeated statement with a spatial power law covariance structure. In the case of a significant interaction, pairwise comparisons between the least squares means of the treatment groups at each time point and between time 0 and each of the other time points within a treatment group were made. In the case where the interaction term was not significant, but time point was significant, pairwise comparisons of least squares means between time 0 and each of the other time points was conducted. The simulate adjustment for multiple comparisons was used for outcomes with significant main effect or interaction terms. The

distribution of the conditional residuals was evaluated for each outcome to ensure the assumptions of the statistical method had been met.

The effect of treatment, group, and time on GI motility was assessed with mixed model logistic regression using PROC GLIMMIX in SAS for Windows v9.4. Period, sequence, treatment group, and time point were included as fixed effects. The interaction of treatment group and time point was included as a fixed effect but was not significant and was removed. Horse within period was included as the subject in a random statement with the residual option to account for repeated measures. An alpha level of 0.05 was used to determine statistical significance for all methods. A mixed model logistic regression analysis was used to determine the odds of decreased gastrointestinal motility between the CRI and IM group.

Results

Pharmacodynamic Data

Normal health status was confirmed by physical examination, complete blood count, and serum chemistry. HR, RR, BG, and NIBP were the pharmacodynamic variables that displayed statistical significance. Decrease in HR was significant in relation to time compared to baseline. Table 1 shows the mean ± standard deviation of HR, RR and BG. At time points 5, 10, 20, 30, 45, 60, 90, 120, 150 and 180 the HR was decreased compared to baseline when accounting for the other effects in the model. Respiratory rate was significant in relation to time compared to baseline. At time points 20, 30, 45, 60, 90, 120, 150 and 180 the RR was significantly decreased compared to baseline when accounting for other effects in the model. Blood glucose was significantly higher in relation to time compared to baseline at time points 30, 60, 90, 120 and 150 when accounting for the other effects in the model.

A group by time interaction was statistically significant for SAP, DAP, and MAP (p-value <0.05). At time points 150 and 180 for SAP, the CRI group had significantly higher values (99 ± 15 mmHg, 98 ± 9 mmHg) when compared to the IM group (82 ± 15 mmHg, 81 ± 11 mmHg) (p-value <0.0232). DAP was significant (p-value < 0.0040) at time point 180 with the CRI group being higher (58 ± 10 mmHg) compared to the IM group (42 ± 7 mmHg). An increase in MAP was statistically significant (p-value <0.0163) at time points 5, 10 and 180 with the CRI (84 ± 12 mmHg, 83 ± 14 mmHg, 73 ± 9 mmHg respectively) group being higher compared to the IM group (68 ± 9 mmHg, 67 ± 8 mmHg, 56 ± 9 mmHg respectively).

Table 2.1 Dexmedetomidine pharmacodynamic data

Time (minutes)	Head Height (cm)		Mechanical Pressure Threshold (kg/cm2)		Heart Rate (beats/min)		Respiratory Rate (f)		Blood Glucose (mg/dL)	
Group	CRI	IM	CRI	IM	CRI	IM	CRI	IM	CRI	IM
Baseline (0)	114 ± 3	111 ± 9	9 ± 2	11 ± 2	42 ± 9	39 ± 6	22 ± 10	19 ± 4	200 ± 44	189 ± 39
5	25 ± 7(*A)	98 ± 18(B)	19 ± 1 (**A)	12 ± 2 (B)	34 ± 4 *	36 ± 4 *	20 ± 5	17 ± 3	189 ± 47	187 ± 37
10	18 ± 10 (*A)	98 ± 16(B)	20 ± 0 (**A)	13 ± 4 (B)	31 ± 4 *	34 ± 4 *	18 ± 4	17 ± 4	193 ± 47	188 ± 48
20	22 ± 6 (*A)	92 ± 16(B)	20 ± 1 (**A)	14 ± 3 (**B)	34 ± 4 *	34 ± 2 *	16 ± 4 *	17 ± 4 *	210 ± 41	198 ± 58
30	33 ± 15 (*A)	70 ± 26(*B)	20 ± 1 (**A)	15 ± 4 (**B)	32 ± 5 *	33 ± 4 *	15 ± 3 *	16 ± 3 *	230 ± 45 **	204 ± 51 **

Table 2.1 (continued)

Time (minutes)	Head Height (cm)		Mechanical Pressure Threshold (kg/cm2)		Heart Rate (beats/min)		Respiratory Rate (f)		Blood Glucose (mg/dL)	
Group	CRI	IM	CRI	IM	CRI	IM	CRI	IM	CRI	IM
45	45 ± 15(*)	59 ± 37(*)	19 ± 2 (**)	16 ± 4 (**)	32 ± 2 *	31 ± 4 *	15 ± 4 *	13 ± 4 *	236 ± 55	205 ± 53
60	48 ± 28(*)	56 ± 27(*)	18 ± 2 (**)	17 ± 3 (**)	33 ± 4 *	32 ± 3 *	15 ± 4 *	14 ± 3 *	239 ± 60 **	227 ± 52 **
90	67 ± 32(*)	47 ± 31(*)	15 ± 4 (**)	17 ± 3 (**)	34 ± 3 *	32 ± 4 *	16 ± 2 *	15 ± 3 *	256 ± 73 **	254 ± 47 **
120	97 ± 17(A)	56 ± 31(*B)	13 ± 4 (**)	15 ± 4 (**)	37 ± 5 *	33 ± 3 *	15 ± 4 *	14 ± 2 *	269 ± 99 **	272 ± 82 **
150	99 ± 12	73 ± 27(*)	11 ± 2	14 ± 4 (**)	36 ± 4 *	36 ± 3 *	17 ± 4 *	14 ± 3 *	264 ± 98 **	280 ± 86 **
180	105 ± 13	77 ± 30(*)	10 ± 3	13 ± 4	36 ± 5 *	36 ± 4 *	15 ± 4 *	18 ± 2 *	253 ± 102	282 ± 105

Mean ± SD for heart rate, respiratory rate, blood glucose, head height, and mechanical pressure threshold values for 8 horses receiving IV and IM dexmedetomidine. * Indicates the time points where heart rate, respiratory rate, and head height were decreased compared to baseline when accounting for other effects in the model. ** Indicates the time points where blood glucose concentration and mechanical pressure threshold were increased compared to baseline when accounting for other effects in the model. A and B represents a significant difference between the groups when accounting for other effects in the model.

Head height demonstrated a group by time interaction (p-value ≤ 0.0028). At time points 5, 10, 20 and 30 there was a significant decrease in head height for the CRI group compared to the IM group. At time point 120 there was a decrease in head height for the IM group compared to the CRI group. These values are shown in Table 2.1 as mean ± standard deviation. A group by time interaction was statistically significant for MPT (p-value <0.0001). At time points 5, 10, 20 and 30 there was an increase in MPT for the CRI group compared to the IM group. These values are

shown in Table 1 as mean ± standard deviation. In a within group comparison in this study for MPT, there was an increase in the threshold at time point 5 and lasted until time point 120 for the CRI group. The IM group had an increase in threshold at time point 20 (14 ± 3 kg/cm^2) and this lasted until time point 150 (14 ± 4 kg/cm^2). A mixed model logistic regression demonstrated that the odds of CRI group would have a decreased motility was 12.3 ($p = 0.0009$) times greater than the IM group. This model determined the odds that GI motility would be depressed with evaluation over 176 time points. Table 2.2 shows the frequency of decreased and normal GI motility scores between the CRI and IM groups. In this study, following IM dexmedetomidine administration, two horses displayed signs of colic approximately 6-8 hours after treatment was discontinued. These clinical signs consisted of decreased appetite, rolling, and laying down in their stalls. A dose of xylazine (150 - 200 mg) and flunixin meglumine (500 mg) were administered and a nasogastric tube was passed and 6L of fluids were administered. The horses were monitored overnight and did not show any additional signs of colic and were able to remain in the study.

Table 2.2 Gastrointestinal motility

	Decreased	Normal	Total
CRI Group	30	58	88
IM Group	6	82	88
Total	36	140	176

Frequency of a decrease in GI motility compared to normal between the CRI and IM group. 176 time points were evaluated and expressed using a contingency table.

Pharmacokinetic Data

Pharmacokinetic parameters for the IV and IM dexmedetomidine plasma concentrations are shown in Table 2 as arithmetic mean ± standard deviation. Assay accuracy was reported as percent nominal concentration and precision as percent relative standard deviation. Accuracy and

precision were 113% and 6% and 110% and 6% for 0.15 ng/mL and 5 ng/mL, respectively. The technique was optimized to provide a lower limit of quantitation of 0.05 ng/mL and a limit of detection of approximately 0.01 ng/mL in plasma. To determine the pharmacokinetic data, a cumulative drug dose of 12,500 ng/kg was used for evaluation of the CRI group and 20,000 ng/kg was used for the evaluation of the IM dexmedetomidine group. The peak plasma concentration for the CRI dexmedetomidine group was 2.44 ± 0.78 ng/ml with the peak plasma concentration occurring at 7.2 ± 5.4 minutes. Peak plasma concentration for the IM dexmedetomidine group was 0.9 ± 0.66 ng/ml. IM peak plasma concentrations occurred at 90 minutes following the first injection. The range was between 45 and 120 minutes. The half-life for the IV group was longer (44.3 ± 26.3 minutes) compared to the half-life for the IM group (38.9 ± 18.6 minutes). Clearance for the CRI group was determined to be 134 ± 67.4 ml/kg/min (CV: 50.3%). Bioavailability for the IM route was estimated as 35 ± 17% when calculated with the $AUC_{0\text{-last}}$ or 37.7 ± 15.6% when calculated with the $AUC_{0\text{-}\infty}$.

Table 2.3 Dexmedetomidine pharmacokinetic data

	Mean	SD	N	Mean	SD	N
Group	CRI	CRI	CRI	IM	IM	IM
AUC[0-last] (min*ng/mL)	109	65.7	8	72.4	68.7	8
AUC[0-inf] (min*ng/mL)	119	63.4	8	81.4	66.1	5
CL (mL/kg/min)	134	67.4	8	.	.	5
Vss (mL/kg)	4890	3920	8	.	.	5
T1/2 (min)	44.3	26.3	8	38.9	18.6	5
Cmax (ng/mL)	2.44	0.778	8	0.895	0.664	8
MRT[0-inf] (min)	35.9	15.4	8	.	.	5
Lambda-z (1/min)	0.02	0.009	8	0.021	0.007	5

Table 2.3 (continued)

	Mean	SD	N	Mean	SD	N
Group	CRI	CRI	CRI	IM	IM	IM
F[0-last] (%)	.	.	.	35.1	17.3	8
F[0-inf] (%)	.	.	.	37.7	15.6	5

Pharmacokinetic mean ± SD for CRI and IM dexmedetomidine plasma concentrations in eight horses. AUC: area under the curve; Cl: clearance (ml kg^{-1} hr^{-1}). V_{ss}: volume of distribution at steady state (ml kg^{-1}). $T_{1/2}$: elimination half-life; Cmax: maximum drug plasma concentration; Tmax: time of maximum drug plasma concentration; MRT: mean residence time; $Lambda_z$: slope; a period indicates that a value was not calculated because it was not applicable.

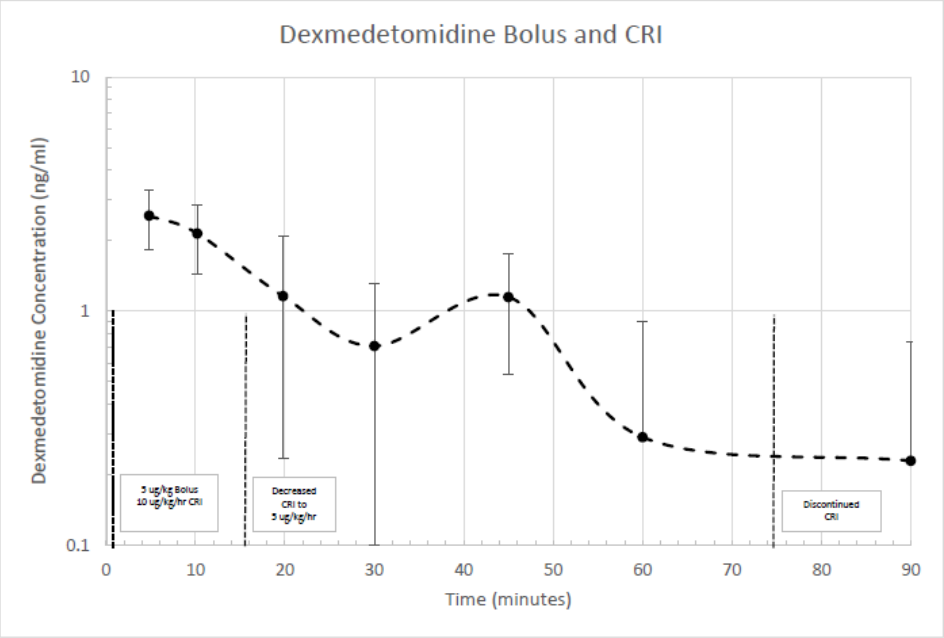

Figure 2.1 Dexmedetomidine plasma concentration following bolus and CRI administration.

Dexmedetomidine plasma concentration ± SD following intravenous administration on a log scale. Time 0 indicates initial bolus dose (0.005 mg/kg) followed by a CRI at 0.01 mg/kg. At time point 15, the CRI was decreased to 0.005 mg/kg. CRI discontinuation occurred at 75 minutes. Dashed line represents concentration-to-concentration connections rather than predicted concentration

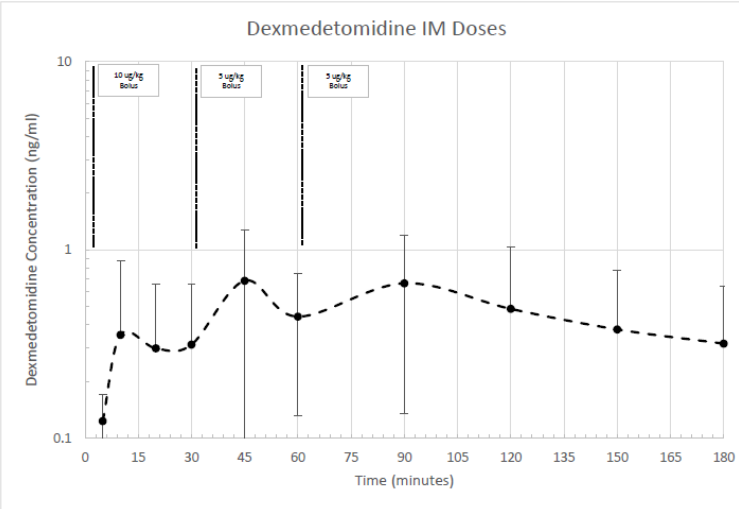

Figure 2.2 Dexmedetomidine plasma concentration following IM administration

Dexmedetomidine plasma concentration ± SD following intramuscular administration on a log scale. Time 0 indicates initial bolus dose (0.01 mg/kg). Additional IM doses occurred at time points 30 and 60. Dashed line represents concentration-to-concentration connections rather than predicted concentration.

Discussion

This study demonstrated that both CRI and IM administered dexmedetomidine produced adequate sedation and increased mechanical pressure threshold that could be potentially useful for equine standing procedures. The CRI group had a faster onset of action with a greater magnitude of sedation and analgesia compared to the IM group. Additionally, the study showed that the odds of a decrease in GI motility were 12.3 times greater for the CRI group compared to the IM group.

Mechanical pressure threshold showed a significant increase in the CRI group (20 ± 1 kg/cm^2) when compared to baseline (9 ± 2 kg/cm^2). MPT as a means to evaluate analgesia has been previously validated using the algometer on the spine of the scapula (Haussler & Erb, 2006; Haussler et al., 2007; Love et al., 2011). This anti-nociceptive property has been shown where

there was a significant increase in MPT for 30 minutes following IV dexmedetomidine administration (Rezende et al., 2015). Dexmedetomidine given as a 2, 4, and 6 μg/kg/hr CRI has been shown to have an anti-nociceptive effect in standing sedation with plasma concentrations ranging from <0.02-0.647 ng/mL (Risberg, et al., 2014). A Tmax of 0.12 ± 0.09 hours explains the quicker onset of action for MPT in the CRI group compared to the IM group. The calculated Tmax for the IM group (45-120 minutes) is consistent with the delayed effects seen in the IM group. In the CRI group, it was demonstrated that at least 2.44 ± 0.78 ng/mL dexmedetomidine plasma level is necessary to produce a significant increase in avoidance threshold for mechanical stimulation. Dexmedetomidine given as a bolus (5 μg/kg) dose produced adequate plasma concentrations (5.7 ± 3.52 ng/mL) to increase avoidance threshold following mechanical stimulation (Rezende, et al., 2014).

Head height has previously been used in studies to evaluate the depth of sedation associated with various anesthetic protocols in horses (Schauvliege et al., 2019). The advantage of HH is that it is a quantitative value and horses are not stimulated for the measurement (Ringer et al., 2013). Although evaluation of HH and its association with sedation is not directly related to surgical procedures, sedation quality is important when considering equine standing surgical procedures. A significant decrease in HH for the CRI group was noted at earlier time points compared to the IM group. Other studies have shown a decrease in head height 10 minutes following an IV detomidine (30 μg/kg) bolus, as well as a decrease in head height for a duration of 60 minutes following a dexmedetomidine IV bolus (Rezende, et al., 2014) (Mama, et al., 2009). When given as a bolus (5 μg/kg), dexmedetomidine has a Cmax of 5.70 ± 3.52 ng/mL with a Tmax (time of maximum plasma concentration) of 2.57 ± 1.40 min (Rezende, et al., 2014). Intravenous

detomidine (30 µg/kg) has been shown to have a Cmax of 105.4 ± 71.6 ng/ml with a Tmax of 1.5 minutes after bolus administration (Mama, et al., 2009). For IM detomidine (30 µg/kg) administration, head height did not decrease significantly until 60 minutes post-drug administration with a Cmax of 6.9 ± 1.4 ng/ml and Tmax of 1.5 hours (Mama, et al., 2009). There is a probable relationship between HH or sedative effect and plasma concentration. With the plasma concentrations obtained in this study, it is possible to predict that with 2.44 ± 0.78 ng/mL sedation will occur. This plasma concentration occurred at 7.2 ± 5.4 minutes. In relation to sedation, the Cmax occurred when HH was significantly decreased compared to baseline between time points 5 and 30 minutes.

As stated previously, this study's CRI group Cmax was higher when compared to the IM group. The Tmax from the initial dose for the CRI group occurred at 7.2 ± 5.4 minutes while the IM group's Tmax was calculated to be between 45 and 120 minutes. Given the dosing regimens used, C_{max} and T_{max} will not generalize to single-dose pharmacokinetics, but they are useful for comparison to pharmacodynamic effects. When looking at the groups individually in relation to HH, there was a difference compared to baseline for CRI starting at time point 5 compared to IM where there was a difference starting at time point 30. Additionally, the CRI group only had a decrease compared to baseline until 90 minutes compared to IM which was significant until time point 180 minutes. Currently, there are no IM dexmedetomidine pharmacokinetic studies to compare these results.

Recent research has shown that a 5 µg/kg bolus of dexmedetomidine produces a Cmax of 5.7 ± 3.52 ng/ml and a CRI of 8 µg/kg/hr of dexmedetomidine produces a Cmax of 0.63 ± 0.44 ng/ml

(Rezende, et al., 2014) (Ranheim, et al., 2014). There are no studies evaluating the pharmacokinetic data of dexmedetomidine when given as an IV bolus followed by a CRI in standing horses. Additionally, Rezende, et al. (2014) and Ranheim, et al. (2014) have shown a large individual variation within pharmacokinetic variables. Due to the previously published C_{max} and variation of dexmedetomidine plasma concentration, this study aimed to verify if an initial bolus of dexmedetomidine (10 µg/kg) followed by a CRI (5 – 10 µg/kg/hr) would allow an initial increase in C_{max} that produced a more consistent steady-state achieved by a CRI. Previous studies evaluating dexmedetomidine given as an IV bolus ranging from 3.5 to 5 µg/kg have determined a half-life of 8 to 28 minutes (Rezende, et al., 2014) (Bettschart-Wolfensberger, et al., 2005). When determining the dexmedetomidine IM intervals, the increase in absorption time and the half-life of IV dexmedetomidine was taken into consideration. From this information, it was concluded that dexmedetomidine IM injection intervals should be 30 minutes to allow for an initial increase in plasma levels followed by a steady-state achieved through intermittent boluses. Noncompartmental analysis was used to evaluate the pharmacokinetics of dexmedetomidine CRI and repeated IM in horses. Similar analysis was previously described by Rezende, et al. (2014) and Ranheim, et al. (2014) for both dexmedetomidine bolus or CRI dexmedetomidine. Some parameters were calculated to be similar and some different from those prior studies; the reasons for the differences are unknown, but could be due to nonlinearity in PK, different sampling intervals, or different PK assay characteristics. These studies showed large variation in pharmacokinetic parameters between individual horses, which also occurred in our study. It is difficult to make comparisons between this study and previous studies due to the variation in doses and dose rates. Ranheim, et al. (2014) calculated the volume of distribution at steady-state (Vd_{ss}) to be 13600 ± 7900 mL/kg with Rezende, et al. (2014) calculating a Vd_{ss} of 1053 ± 472.58

mL/kg. In our study, the Vd_{ss} was determined to be 4890 ± 3920 mL/kg. Similar to Ranheim, et al. (2014) CRI study, our study reveals a coefficient of variation for Vd_{ss}. Additionally, the mean falls between the values found in Ranheim, et al. (2014) and Rezende, et al. (2014). Variations in Vd_{ss} will occur due to proportionality between the amount of drug in the body (mg) and plasma concentration (mg/L). This could be explained by Ranheim, et al. (2014) administering a CRI of 8 µg/kg/hr and Rezende, et al. (2014) administering one bolus dose of 5 µg/kg without any additional drugs. Clearance for the IV group (7168 ± 3622 ml/kg/hr) was decreased compared to Ranheim, et al. (2014) (26400 ± 16200 ml/kg/hr) and prolonged compared to Rezende, et al. (2014) (4717.2 ± 3598.2 ml/kg/hr). All these values have large coefficients of variation. Previous research has shown that with an increase in plasma dexmedetomidine concentration, there is a decrease in cardiac output that leads to a decrease in clearance (Dutta et al., 2000); this nonlinearity could be responsible for some differences in calculated parameters. Dexmedetomidine given as a bolus (3.5 µg/kg) in horses has shown a similar effect with a decrease in stroke volume and cardiac index following administration (Bettschart-Wolfensberger, et al., 2005). This decrease in cardiac output following dexmedetomidine administration could explain the individual variability in plasma clearance and half-life. Half-life for the CRI group was 44.3 ± 26.3 minutes and 38.9 ± 18.6 minutes for the IM group. Ranheim, et al. (2014) reported the half-life to be 20.4 ± 6 minutes for CRI administration without an initial bolus dose. In this study, a bolus (0.005 mg/kg) was administered prior to starting the CRI. The other pharmacodynamic variables that were evaluated were HR, RR, BG and NIBP. These variables were measured to determine if there were adverse effects of dexmedetomidine administration. The parameters that changed significantly in relation to time were HR, RR, and BG. It was anticipated that HR and RR would be decreased in comparison to baseline due to

previous dexmedetomidine research (Rezende, et al., 2014). BG was increased at multiple time points compared to baseline. This was an expected outcome as alpha-2 adrenergic receptor agonists directly inhibit the release of insulin causing an increase in blood glucose levels (Gertler, et al., 2001). NIBP changed significantly with a group by time interaction. For this study, the blood pressure cuff was not corrected to heart level. A measurement at the coccygeal artery without correction to the base of the heart will cause an underestimation in blood pressure in standing horses (Heliczer et al., 2016). For this study, correction was not applied to the NIBP values as an overall trend was being evaluated. For precise measurements of BP, invasive monitoring would have to be used. Due to the nature of the study, invasive BP measurements would have increased cost, time, and stress to the horses.

Two horses had clinical signs of colic following IM administration. These horses were allowed to remain in the study as xylazine has a short duration of action and half-life and would not affect pharmacodynamic or pharmacokinetic parameters following the 3-day washout period. Flunixin meglumine also has a short duration of action that would not affect the study parameters following a 3-day washout period (Grimm, et al., 2015). A decrease in GI motility was not unanticipated, due to well documented effects of alpha-2 adrenergic receptor agonists on GI motility. Pre- and post-synaptic alpha-2 adrenoceptors are present throughout the gastrointestinal tract that have an inhibitory effect on motility when alpha-2 agonists are utilized (Zullian, et al., 2011). Previously, a decrease in borborygmi values for 60 minutes was noted following a bolus of 5 µg/kg dexmedetomidine IV (Rezende, et al., 2014). Detomidine given at 30 µg/kg bolus, both IM and IV, decreased GI motility (Mama, et al., 2009). This decrease had a greater magnitude and onset in the IV group compared to the IM group (Mama, et al., 2009). When

comparing plasma concentrations in this study, the CRI group had a higher Cmax (maximum plasma concentration) (2.44 ±0.78 ng/mL) while the IM group's Cmax (0.90 ± 0.66 ng/mL) was lower. Due to the higher plasma concentrations in the CRI group, it would have been anticipated to see more colic symptoms in this group compared to the IM group. When looking at table 3, the IM group had a 16.7% chance of having a decrease in GI motility compared to an 83.3%seen in the CRI group. Further research will be needed to evaluate the effects of IM dexmedetomidine on GI motility.

Our study presents some limitations. First, although sample size was properly calculated, there were a relatively small number of horses utilized for the study which could have amplified the effects of individual variation in drug pharmacokinetics. The sample size calculations assumed a difference of 10 ng/mL between groups and that difference was not achieved. A minimum difference of 10 ng/mL between groups was chosen due to the original bolus dose administered followed by either a CRI or intermittent IM injections. These doses were higher compared to previous studies and it was anticipated that a 10 ng/mL difference would occur between the two groups. Second, a control group without dexmedetomidine administration was not utilized. In future studies, having a control group would allow comparison of pharmacodynamic data between control horses and horses in both the IV and IM group. Third, for both the IV and IM groups, there were data points following dexmedetomidine administration that plasma concentrations were lower than the LOQ. Due to plasma concentrations below the LOQ at the time points following CRI discontinuation, it was not possible to calculate half-life for all animals. In future studies, inclusion of more data points following discontinuation of the CRI and before the LLOQ is reached would allow a more accurate half-life and clearance to be calculated.

The current T_{max} that has been calculated occurred after the initial bolus dose. For this study, it was anticipated that the bolus dose would accomplish the desired dexmedetomidine plasma concentration and the CRI would allow a steady state. It can be seen in Figure 1 that the CRI did not accomplish a steady state and there was a steady decline in the dexmedetomidine plasma concentration.

Our first hypothesis was that there would be a difference in the dexmedetomidine plasma concentration of 10 ng/ml between the CRI and IM group. The second hypothesis was that the difference in dexmedetomidine plasma concentration would lead to changes in the pharmacodynamic parameters. In relation to HR, RR and BG, both the CRI and IM groups had similar values. When comparing HH and MPT, the CRI group had a faster onset of action compared to the IM group.

In the future, this information could be applied to standing surgical procedures in horses. This would decrease the need of placing horses under general anesthesia. This study evaluated the pharmacokinetics and pharmacodynamics of dexmedetomidine using a CRI group (IV bolus of dexmedetomidine at 0.005 mg/kg followed by a CRI at 0.01 mg/kg/hr for 15 minutes then 0.005 mg/kg/hr for 60 minutes) and an IM group (dexmedetomidine at 0.01 mg/kg, followed by 0.005 mg/kg in 30-minute intervals for 60 minutes). Additionally, even though the IM group had a delayed onset of sedation and analgesia, it is possible, that with further research, it may be used in place of a CRI to produce similar effects. The anesthetist would need to recognize that these effects would have a delayed onset of action of 30 to 45 minutes following IM administration. In conclusion, dexmedetomidine, given as both a CRI and IM, produces adequate sedation and an

increase in MPT in standing horses. Minimum plasma levels of dexmedetomidine necessary to increase avoidance threshold to mechanical nociceptive stimulation is 2.44 ± 0.78 ng/mL.

CHAPTER III

CONCLUSION

Dexmedetomidine is used for equine sedation, analgesia, and as an adjuvant to general anesthesia. A dexmedetomidine IV bolus (0.005 mg/kg) followed by a CRI (0.01 mg/kg/hr for 15 minutes then 0.005 mg/kg/hr for 60 minutes) shows an increase in mechanical pressure threshold with an earlier onset and greater magnitude compared to IM dexmedetomidine (0.01 mg/kg, followed by 0.005 mg/kg in 30-minute intervals for 60 minutes). Similar effects are seen when evaluating sedation through head height. When evaluating other pharmacodynamic data such as HR, RR, and BG, these values had similar results between groups.

Evaluation of dexmedetomidine pharmacokinetics through the non-compartmental approach yielded various pharmacokinetic parameters that are shown in Table 2.3. This table shows a difference in the C_{max} between the CRI (2.44 ± 0.778 ng/ml) and IM (0.895 ± 0.664 ng/ml) group. The original hypothesis of this study was that there would be a difference in dexmedetomidine plasma levels of 10 ng/ml between the CRI and IM group and this hypothesis was rejected. Half-life between the CRI (44.3 ± 36.3 minutes) and the IM (38.9 ± 18.6 minutes) groups were similar. Through non-compartmental analysis, a clearance (134 ± 67.4 mL/kg/min) and V_{ss} (4890 ± 3920 mL/kg) of the CRI group were able to be determined.

Previous research has determined that dexmedetomidine exhibits a large variability in plasma concentrations (Ranheim et al., 2015; Rezende et al., 2015). One possible explanation for this variability is through a partitioning effect between the plasma and red blood cells. A 100 µg/kg IV bolus dose of romifidine has been shown to have a partitioning effect with red blood cells causing a consistent and continuous release of romifidine from the cells to the plasma (Romagnoli et al., 2017). Previous research indicates that certain drugs will bind to proteins in the red blood cells and these drugs will move by passive diffusion from the red blood cells and into the plasma (Hinderling, 1997). No research has been done examining dexmedetomidine and a possible partitioning effect. Future studies are required to evaluate whether dexmedetomidine has a similar partitioning effect that is causing large variability in plasma concentrations due to sequestration within the red blood cells.

Noncompartmental analysis was utilized for the CRI and IM group due to lack of data points available to determine the terminal half-life. Future studies will need to be utilized to incorporate more data points following discontinuation of the CRI in the CRI group. Having more data points after discontinuation of the CRI may allow more dexmedetomidine plasma levels higher than LOQ. If a terminal half-life is able to be calculated, compartmental analysis may be utilized and a dosing regimen can be determined.

Future studies may include evaluation of GI motility and ataxia scores following CRI and IM administration of dexmedetomidine. This study shows that the odds of decreased GI motility through auscultation was 12.3 times greater in the CRI compared to the IM group. In this study, following IM dexmedetomidine administration, two horses displayed signs of colic. Additional

studies can be utilized to evaluate varying IM dexmedetomidine doses and the effects on GI motility in relation to sedation and analgesia scores. A dexmedetomidine IV bolus (0.005 mg/kg) produced reluctance to walk with ataxia at four and ten minutes after administration (Rezende et al., 2015). The current study did not include ataxia scores due to time and space limitations. In the future, determination of ataxia scores following CRI and IM dexmedetomidine will be useful for standing surgical procedures.

This study evaluated the pharmacokinetics and pharmacodynamics of CRI and IM dexmedetomidine in standing horses. In conclusion, dexmedetomidine, given as both a CRI and IM, produces adequate sedation and an increase in MPT in standing horses.

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