Review Article

Effects of Yohimbine Over Pharmacokinetic and Pharmacodynamic and Behavioral Parameters in Horses Sedated With Detomidine

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A B S T R A C T

The aim of this study was to review the safety of the detomidine (DET) in horses and the effects of yohimbine (YOH) over pharmacokinetic, pharmacodynamic, and behavioral parameters in horses sedated with DET. A literature search was made on PubMed (National Center for Biotechnology Information, United States National Library, Bethesda, MD) and SCOPUS (Elsevier Research Intelligence) for studies that had evaluated the effects of DET or YOH on clinics pharmacodynamics and pharmacokinetics parameters in horses plus experimental studies with the effect of YOH on the pharmacokinetics, pharmacodynamics, and behavioral parameters in horses sedated with DET. Additionally, information was obtained from studies where DET or YOH was administered alone or in their combination in treatment of horses. Three investigations described the pharmacokinetics or physiologic effects of YOH when administered after DET to reverse the behavioral and physiologic effects of DET. The studies with DET showed that it was more absorbed when administered intramuscular than when administered sublingual. In those studies, they noted important implications, both from therapeutics and regulatory prospective. They demonstrated intravenously administered DET is effective in sedation with effects on cardiovascular effects.

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1. Introduction

There is a wide group of alpha-2 adrenergic adrenoreceptor agonists such as xylazine, detomidine (DET) (DET is alpha-2 adrenergic adrenoreceptor agonists), medetomidine, and romifidine. In veterinary practice, xylazine and medetomidine are the most commonly used drugs for horses. DET is a potent agonist of both centrally and peripherally located alpha-2 receptors in many animal species [1,2] and is characterized by rapid distribution and metabolism to two main metabolites with subsequent elimination [3]. DET is commonly used in equine medicine for procedures requiring sedation, chemical restraint, or analgesia and is most commonly administered parenterally [3–7]. The effects of DET on the pharmacokinetics and pharmacodynamics parameters in the horse following either intravenous (IV) or intramuscular (IM) administration have been well described [2,8–11].

Alpha-2 adrenergic antagonists are often used to reverse the sedative, cardiovascular depressant [3] and central nervous system (CNS) effects of alpha-2 adrenergic receptor agonists following IV or IM administration. The three antagonist most commonly used in veterinary

medicine are yohimbine (YOH) (alpha-2 receptor antagonist), atipamezole, and tolazoline. In equine medicine, the only Food and Drug Administration (FDA) approved alpha-2 adrenergic antagonist is tolazoline [1]. Yohimbine is an indole alkaloid derived from several biological or botanical sources, including the bark of the *Pausinystalia yohimbine* tree and the Rauwolfia root [2,3]. Yohimbine enhances sympathetic outflow via the neurotransmitter, norepinephrine. It is a potent antagonist of centrally and peripherally located alpha-2 receptors in humans and many animal species [12–14]. In veterinary medicine, YOH is almost exclusively used to reverse the sedative or cardiovascular effects of the alpha-2 receptor agonists, especially DET [2]. In horses, YOH has been shown to antagonize the ventricular bradycardia and atrioventricular (AV) conduction disturbances observed following administration of DET [3]. Yohimbine appears to be widely distributed as evidenced by a large volume of distribution and rapidly cleared following IV administration to the horse [5]. In the human, YOH is rapidly metabolized by the cytochrome P450 enzymes to two hydroxyl-yohimbine metabolites [15]. To our knowledge, there are no reports in the literature regarding YOH metabolites in the horse [5]. Hydroxylation is the major pathway for elimination of YOH in the horse. However, although hydroxylation of YOH in humans has been attributed to CYP450 enzymes, namely CYP3A4 and CYP2D6, the identity of the enzymes responsible for metabolism of YOH in the horse has yet to be elucidated [5]. Based on the evidence of experimental studies on its efficacy, the aim of this study was to systematically review the safety of the drug in horses and the effects of YOH over pharmacokinetic, pharmacodynamic, and behavioral parameters in horses sedated with DET.

2. Methods

A literature search was made on PubMed (National Center for Biotechnology Information, United States National Library, Bethesda, MD) and SCOPUS (Elsevier Research Intelligence) from its inception on May 26, 2015. In the review, experimental studies involving the evaluation of the effects of DET administered enterally or parenterally in horses on clinics pharmacodynamics and pharmacokinetics parameters were included. Experimental studies that determined the pharmacokinetics or pharmacodynamics profile of intravenously administered YOH in horses were also included. Finally, experimental studies evaluating the effect of YOH on the pharmacokinetics, pharmacodynamics, and behavioral parameters in horse sedated with DET were included. A review of titles and, if available, abstracts was performed by two of the investigators who eliminated duplicate manuscripts and studies evaluating the effects of other alpha-2 adrenergic antagonists on horse. Five manuscripts were retrieved for further revision. Disagreements between the investigators were resolved by consensus.

Data abstraction was performed by three other investigators. From the experimental studies performed in horses, the following variables were obtained: animal species, sex, age, dosage, administration route, clinics effects, changes in behavior, cardiac and blood parameters, pharmacokinetics and pharmacodynamics effects. Of the 26 retrieved studies, the information was obtained from 14 selected reports [1,3–7].

3. Results

The following studies reporting treatments in horses which employed DET or YOH when administered alone or in combination were identified. Three in vivo experimental studies with horses characterized pharmacokinetics, pharmacodynamics, sedative, and clinical effects of DET. The DET was administered at different doses enterally or parenterally. DET doses of 0.03 mg kg^{-1} was most frequently chosen for two reasons, it is the dose commonly used for sedation in horses, and this dose has demonstrated the minimum effects on alveolar concentration of isoflurane in horses [7]. However, studies with this drug do not use this suggested dose. The first study characterized the pharmacokinetics of a novel DET gel product after sublingual (SL) administration indicated slight differences in absorption and plasma DET concentrations. Carboxydetomidine and hydroxydetomidine were detected in urine samples. The elimination of DET differed between sedentary and active horses. For the second experiment, area under the curve and maximal plasma concentration (C_{max}) showed that IM and SL routes of administration were not bioequivalent. The onset of sedation was very fast with IV administration. However, the time to the onset of sedation was longer after SL and IM administration. Part of the gel is likely to be swallowed and, due to extensive first-pass metabolism, does not reach the systemic circulation. In two experiments, no adverse effects were observed in horses that were treated via SL. Other study showed the pharmacokinetics parameters of DET where the clearance was considerably faster and the volume of distribution markedly higher compared to previous reports in the some specie (Table 1).

Three experimental studies characterized the pharmacokinetics or pharmacodynamics profile and determine the half-life of YOH when administered to horses. The studies were conducted in a randomized fashion at different doses administered intravenously where in each horse received 0.075, 0.1, 0.12, 0.15, 0.2, or 0.4 mg/kg of YOH. Mean plasma YOH concentration in the first 15 minutes following IV administration of 0.4 mg/kg YOH corresponded to 105 or 220 ng/mL (Table 2). Immediately following administration, some horses showed signs of sedation which persisted for approximately 1 hour, as indicated by a slight drop in head height (chin-to-ground distance). Gastrointestinal (GI) sounds increased in most horses at all doses studied; nevertheless, a dose-dependent response was evident with GI sounds.

Another three investigations described the pharmacokinetics or physiologic effects of the YOH when administered after the DET to reverse the behavioral and physiologic effects of DET. The experimental studies with DET showed that DET had been absorbed when administration route was SL but was less absorbed than when given IM. In these studies (references), the authors noted important implications, both from therapeutics and regulatory prospective. These studies demonstrated that intravenously administered DET was effective in sedation, but with negative effects on cardiovascular (Table 3).

Table 1
Effects of detomidine in horses on clinics, pharmacodynamics, or pharmacokinetics parameters.

Authors	Objective	Experimental Model	Dose and Administration Route	Pharmacokinetics or Pharmacodynamics Changes	Conclusion
di Maio Knych and Stanley, 2011	Characterize pharmacokinetics and pharmacodynamics of DET gel administered in horses before competition.	Twelve healthy fit actively competing adult Thoroughbred racehorses were included. Horses were assessed as healthy and free of cardiovascular disease. Horses did not receive any sedative or analgesic agents; they continued to be exercised throughout the sample collection period, except for the day of drug administration, during which no exercise was performed.	0.04 mg/kg DET (Dormosedan Gel, Pfizer Animal Health, New York, NY) administered SL.	Highest plasma DET concentration was 168 ± 83.7 ng/mL. Peak DET plasma concentration was rapidly with mean $\pm$ SD time of maximal plasma concentration (Tmax) at 36 ± 10 min after drug administration. Half-life of elimination was 1.5 ± 1 h. Concentrations of DET as well as its metabolites in urine samples were below the limit of detection limit of detection (LOD) by 3 days after administration.	DET gel appeared to have been absorbed well from the SL mucosa into the systemic circulation. The half-life of elimination following SL administration was prolonged, compared with IV or IM administration, with detectable concentrations of DET or its metabolites in plasma for up to 24 h after administration.
Kaukinen et al, 2011	Determine the absorption, bioavailability, and sedative effect of DET administered to horses as an oromucosal gel compared to IV and IM administration DET injectable solution.	Nine healthy horses (five Standardbreds and four warmbloods) were used. Each horse was allocated by computer-generated randomization to receive DET via each route in a randomized order. Blood samples were collected before and after drug administration for the measurement of DET concentrations in serum. Pharmacokinetic variables were estimated for each horse and each dosing occasion.	40 µg/kg DET route IV, IM or administered under the tongue with a 7-d washout period between treatments. DET was given as a bolus into the jugular vein, as an IM injection into the neck muscles (Domosedan 10 mg/mL solution, Orion Pharma, Espoo, Finland) or as an oromucosal gel (Domosedan Gel 7.6 mg/mL, Orion Pharma, Turku, Finland).	Slow absorption leads to fewer and less pronounced adverse effects than the more rapid absorption after IM injection. Maximum concentration for DET given via SL route was lower than following IM administration (geometric mean 4.16 vs. 11.16 ng/mL) and the t_{max} was longer (1.83 vs. 1.06 h).	Less DET is absorbed when given SL than when given IM because part of it does not reach the circulation. Sublingual administration of DET oromucosal gel at 40 µg/kg produces safe sedation in horses. Slow absorption leads to fewer and less pronounced adverse effects than the more rapid absorption after IM injection.
Vainionpää et al, 2013	Investigate plasma drug concentrations and the effect of the peripherally acting alpha-2-adrenoceptor antagonist MK-467 (L-659′066) on sedation, HR, and gut motility in horses sedated with IV DET.	Six healthy Finnhorse mares were used. They were not pregnant and in winter anoestrus. The depth of sedation, intestinal sounds, attitude, posture, height of the head, eyelid aperture, and movement of the ears were scored before and after treatment. An electrocardiogram was recorded continuously, HR and rhythm were evaluated before and after of the injection. Blood was collected after drug administration.	10 µg/kg^{-1} DET (Equisedan, Vetcare, Finland) was administered IV alone and in combination with MK-467 250 µg/kg^{-1} (Merck & Co, Inc, NJ) IV in a randomized, crossover design with a minimum of 14 d between treatments.	AUC_{sed} was significantly higher with DET than DET + MK-467, but maximal sedations scores did not differ significantly between treatments. MK-467 lowered the AUC of the plasma concentration of DET and increased its volume of distribution and clearance. A significant reduction in HR was detected after DET. HR was significantly higher after DET-MK-467 than DET. DET-induced intestinal hypomotility was prevented by MK-467.	MK-467 prevented DET-induced bradycardia and intestinal hypomotility. MK-467 did not affect the clinical quality of DET-induced sedation, but the duration of the effect was reduced, which may have been caused by the effects of MK-467 on the plasma concentration of DET. MK-467 may be useful clinically in the prevention of certain peripheral side effects of DET in horses.

Abbreviations: AUC, area under the curve; DET, detomidine; HR, heart rate; IM, intramuscular; IV, intravenous; SD, standard deviation; SL, sublingual.

Table 2
Effects of yohimbine in horses on clinics, pharmacodynamics, or pharmacokinetics parameters.

Authors	Objective	Experimental Model	Dose and Administration Route	Pharmacokinetics, Pharmacodynamics, or Behavioral Changes	Conclusion
Dimaio Knych et al 2011	Determine the pharmacokinetic profile of IV-administered YOH in horse.	Eight healthy unmedicated adult horses including seven thoroughbreds and one Standardbred. Blood samples were collected prior and at various times up to 72 h postdrug administration. Data analyzed using both noncompartmental and compartmental analysis.	0.12 mg/kg YOH (Yobine, Lloyd Laboratories, Shenandoah, IA) an IV dose administered slowly over 1 min.	Peak plasma concentration was 114.5 ± 31.8 ng/mL, occurred at 0.09 ± 0.03 h. Systemic clearance and steady-state volume of distribution were 13.5 ± 2.1 mL/min/kg and 3.3 ± 1.3 L/kg following noncompartmental analysis. Terminal elimination half-life was 4.4 ± 0.9 h. For compartmental analysis, plasma YOH versus time data were best fitted to a two-compartment model. Systemic clearance and steady-state volume of distribution of YOH were 13.6 ± 2.0 mL/min/kg and 3.2 ± 1.1 L/kg.	They were able to detect YOH in plasma samples at 12 h postdrug administration, suggesting that YOH is characterized by prolonged elimination, most likely as a result of sequestration and slow release over time. A dose of 0.12 mg/kg IV to horse has a large volume of distribution. Large volume of distribution coupled with slower systemic clearance determined supports the longer terminal elimination half-life.
Knych et al [3]	Investigate the pharmacokinetics and pharmacodynamics of YOH when administered IV to horse.	Nine healthy unmedicated adult horses including 8 Thoroughbreds and 1 Standardbred A minimum of 1 wk was allowed to elapse between administrations of additional doses to the same horse. Blood samples were collected prior and at various times up to 24 h postdrug administration and were analyzed using liquid chromatography–mass spectrometry. Data analyzed using both noncompartmental and compartmental analysis.	0.1, 0.2, and 0.4 mg/kg YOH (Yobine; Lloyd Laboratories, Iowa), IV administered slowly over 1 min.	Peak plasma concentration was 106.0 ± 28.9, 156.7 ± 34.3 and 223.0 ± 44.5 ng/mL for doses of 0.1, 0.2, and 0.4 mg/kg, occurred at 0.09 ± 0.03 h. Systemic clearance and steady-state volume of distribution were 12.0, 12.2, and 17.9 mL/min/kg and 2.1, 2.6 and 2.9 L/kg following noncompartmental analysis. Terminal elimination half-life was 43.6, 3.3, and 2.9 for doses of 0.1, 0.2, and 0.4 mg/kg. For compartmental analysis, plasma YOH versus time data were best fitted to a two-compartment model. Systemic clearance and steady-state volume of distribution of YOH were 11.1 mL/min/kg and 2.3 L/kg.	Behavioral responses following YOH administration are highly variable between horses. Yohimbine had profound effects on heart rate and rhythm, with maximal heart rates exceeding 100 beats/min in some horses. YOH should be used with caution as there is the potential for unpredictable harmful effects.
Hubbell et al [9]	Characterize the pharmacokinetics profile and determine the half-life of YOH with two dosages in horses.	Two groups of horses (11 Crossbred horses; 7 geldings; and 4 mares) were used to determine whether the half-life varied when the dose was changed. Blood samples were collected prior and at various times up to 3 h postdrug administration. For pharmacokinetic analysis, a noncompartmental approach using statistical moment theory was used.	YOH hydrochloride (Sigma Chemical Co, St. Louis, Missouri) was prepared as a 0.4% wt/vol. 0.075 (seven horses) or 0.15 (four horses) mg/kg was injected into the apposite jugular vein.	No significant differences in any of the pharmacokinetic parameters between doses of YOH. Systemic clearance and steady-state volume of distribution of YOH were 39.6 ± 16.6 versus 34.0 ± 19.4 mL/min/kg and 4.6 ± 1.9 versus 2.7 ± 1.0 L/kg in horses given doses. The mean effective half-life for YOH was 76.1 ± 23.1 min and 52.8 ± 27.8 min in horses given small or large doses of YOH.	The large volume of distribution, due to YOHs lipid solubility and ability to cross membranes was seen. Their results indicated relatively long serum half-live of YOH in horses.

Abbreviations: IV, intravenous; YOH, yohimbine.

The behavioral effects of the alpha-2 receptor antagonist, YOH, appeared to be highly variable between horses. Regardless of the variability in response when administered alone, YOH was effective in resolving naturally occurring as well as DET-induced AV conduction disturbances.

4. Discussion

In relation to administration of DET in horses, it has been shown individual variability in pharmacokinetics parameters can be attributed to factors such as dose, loss of drug, dose lost via expulsion from the mouth or swallowing, or DET metabolism by enzymes in the GI tract wall or first-pass effects [8]. DET is a lipophilic weak base with an acid dissociation constant (Pka) of 7.2; thus, absorption is favored in an alkaline environment, mouth and small intestine included. Oral cavity in horses tends to be alkaline, which makes it possible that slight differences. Sublingual administration of DET gel was well tolerated by horses, barley perceptible diffuse erythema of oral mucous membranes was reported in horses 4 to 6 hours after treatment [4], and this reaction may be a consequence of head drooping during sedation. The researchers [4] reported that the bioavailability of DET administered as a oromucosal gel was about 22% versus 38% of the IM formulation. Salonen et al [2] indicated that due to extensive first-pass metabolism, the drug does not reach the systemic circulation. Sublingual administration of DET is apt to reach the heart before distribution to the brain because the mucosal capillaries drain directly into the jugular veins, which run directly to the heart. However, blood must travel throughout the body before reaching the brain [8].

The highest plasma DET concentration was 168 $\pm$ 83.7 ng/mL, which indicates that the drug was absorbed well from the SL mucosa into the systemic circulation. In SL administration, the drainage from the submucosal region was via the jugular vein. In this respect, it is important to know whether if collection of samples was via a jugular vein immediately following absorption. Although DET appeared to be absorbed well following SL administration, there was a great degree of variability in C_{max} and T_{max} among horses. The C_{max} differed substantially as a result of the site used for collection of samples and the time after drug administration [8].

The elimination of drugs has been reported to differ between sedentary and active horses [8]. Previous studies have reported the elimination half-life of DET to be 26 to 71 minutes [5]. Knych and Stanley [6] reported a half-life of 37 minutes, resulting in observations analogous with the previously reported findings. The mean half-life of elimination of DET following SL administration reported by Grimsrud et al [8] was 1.5 $\pm$ 1 hours and longer than after IV administration which was 26.4 minutes. Terminal half-life of DET was longer after SL than after IM administration, but sedation lasted longer after IM administration [4]. This can be explained by the lesser bioavailability of DET as a oromucosal gel, compared to the injectable solution, reflecting the dose-dependent duration of DET sedation [16].

DET administration of SL and IV produced profound sedation in all horses studied as evidenced by an observable decrease in chin-to-ground distance [7]. Kaukinen et al

(2011) reported that sedation started sooner after IM administration than after the administration of the oromucosal gel. This can be explained by the lower mean C_{max} and the longer mean t_{max} after SL administration via IM injection, indicating that DET is absorbed more rapidly when given IM to horse [4]. DET produces cardiovascular side effects and ataxia [17], in the study of Kaukinen et al, 2011; however, those effects were less pronounces after the SL administration. No adverse effects were observed in the oromucosal gel group, with the exception of only adverse effect after IM treatment was mild bradycardia observed in one horse [4]. It has been shown that some horses exhibit signs of ataxia (stable but swaying lightly) between the 40- to 90-minute assessment points after oromucosal gel administration. The bradycardia and conduction disturbances observed following DET administration may be attributable to a centrally mediated decrease in peripheral sympathetic tone, presynaptic inhibition of norepinephrine release from fibers innervating the heart, or enhancement of vagal reflexes [11].

A distinct advantage of alpha-2 adrenergic agonists, such as DET, is the availability of antagonists that can revert the pharmacologic effects of alpha-2 agonists, whose antagonists are beneficial in cases of overdoses of alpha-2 adrenergic agonists [1]. Several studies have previously reported the pharmacokinetics of DET, YOH, and DET-YOH [5], and other studies have described some pharmacodynamics effects of DET followed by administration of YOH [7] or have characterized behavioral parameters in horses sedated with these drug combinations.

Dimaio Knych et al [17] noted a marked increase in glucose concentrations 30 minutes post-DET. Hyperglycemia has been detected in horses [6,17] and has been attributed to inhibition of insulin release from the pancreas beta cells. di Maio Knych and Stanley (2011) reported no apparent pattern for glucose concentrations over the 6-hour sample collection period although there was a large variability among horses [18] suggesting the possibility of a non-drug–related phenomenon and simply a result of food being withheld from the horses before and throughout the glucose-monitoring period. There is need for additional studies to characterize these effects in horses. In relation to administration of YOH, it has been shown that there are individual variation in the pharmacokinetics parameters. It is possible that the differences observed were caused by age, physical condition, intrinsic clearance, amount of body fat, and tissue blood flow [9]. The pharmacokinetic parameters calculated for YOH as the large volume of distribution was due to its rapidly dispersed (2.0–5.7 L/kg). The lipid solubility and lipophilic compound of YOH may allow it to cross the blood-brain barrier to a potential site of action in the CNS. The same researchers indicated extensive tissue distribution and ability to cross membranes, which helps to explain its duration and action when used for arousal from anesthesia.

The mean half-life of YOH was 86.6 minutes in horses given a small dose of 0.075 mg/kg and 57.8 minutes in horses given a large dose of 0.15 mg/kg [9]. The relatively long serum half-life and mean residence time of YOH indicated that this would be present in the body until after most anesthetics or sedatives were no longer effective. In

Table 3

Effects of yohimbine on changes in behavior, pharmacodynamics, or pharmacokinetics parameters of detomidine in the horse.

Authors	Objective	Experimental Model	Dose and Administration Route	Pharmacokinetics, Pharmacodynamics, or Behavioral Changes	Conclusion
Heather K. Dimaio Knych, et al, 2012	Describe pharmacodynamics effects of DET and YOH when administered alone and in sequence.	Nine adult horses (eight Thoroughbreds and one Standardbred). Blood samples were obtained prior and at various times up to 72 h postdrug administration. Plasma samples were analyzed using liquid chromatography–mass spectrometry. Behavioral effects, heart rate and rhythm, glucose, packed cell volume (PCV), and plasma proteins were monitored.	Three dose regimens were employed. (1) 0.03 mg kg^{-1} DET (Dormosedan, Pfizer Animal Health, PA) IV. (2) 0.2 mg kg^{-1} YOH (Yobine, Lloyd Laboratories, IA) IV. (3) 0.03 mg kg^{-1} DET IV followed 15 min later by 0.2 mg kg^{-1} YOH IV. Each horse received all three treatments with a minimum of 1 wk between treatments.	Heart rate decreased significantly for all horses, following DET administration. The maximal decrease (15 bpm) was present 2 min post-DET. Bradycardia persisted for up to 1 h post-DET administration. Yohimbine returned heart rate and the percent of AV conduction disturbances to pre-DET values when administered 15 min post-DET. Plasma glucose concentrations increased by 30 min post-DET administration, both for the DET only and the DET + YOH dose groups (44 and 32 mg/dL^{-1}).	DET is effective in inducing sedation with pronounced effects on cardiac effects, including a notable decrease in heart rate and an increased incidence of AV conduction blocks. IV administration of YOH is effective in reversing the behavioral and cardiovascular effects of IV-administered DET. Yohimbine induced sedation, bradycardia, AV heart block, and hyperglycemia.
Knych et al, 2012	Describe the pharmacokinetics of DET and YOH when administered in combination.	Nine adult horses (eight Thoroughbreds and one Standardbred) were studied. Each horse received all three dose regimens with a minimum of 1 wk in between subsequent regimens. Blood samples were collected at time 0 (immediately prior to DET administration) and at 1 h post-DET administration (immediately prior to antagonist administration) and at 5, 10, 15, 30, 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 18, 24, 36, 48, and 72 h postadministration of the DET and YOH treatment. Plasma was analyzed for DET and YOH concentrations by liquid chromatography–mass spectrometry. Data were analyzed using both noncompartmental and compartmental analysis.	Three dose regimens were employed. (1) 0.03 mg kg^{-1} DET (Dormosedan, Pfizer Animal Health, PA) IV. (2) 0.2 mg kg^{-1} YOH (Yobine, Lloyd Laboratories, IA) IV. (3) 0.03 mg kg^{-1} DET IV followed 15 min later by 0.2 mg kg^{-1} YOH IV.	The Cl system and V_d of DET were not significantly different for either treatment. The maximum measured DET concentrations were 76.0 and 129.9 ng mL^{-1} for the DET and DET-YOH treatments, respectively. Systemic clearance and V_d of DET were not significantly different for either treatment. There was a significant increase in the maximum measured YOH plasma concentrations from YOH (173.9 ng mL^{-1}) to DET-YOH (289.8 ng mL^{-1}). Both the Cl and V_d for YOH were significantly less (6.8 mL minute^{-1} kg^{-1} and 1.7 L kg^{-1}) for the DET-YOH as compared to the YOH treatments (13.9 mL minute^{-1} kg^{-1} and 2.7 L kg^{-1}).	DET increases plasma YOH concentrations and decreases the Cl and V_d compared to administration of YOH by itself. The elimination half-life of YOH remained unaffected when administered subsequent to DET. However, the increased plasma concentrations in the presence of DET has the potential to cause untoward effects, and therefore, further studies to assess the physiologic effects of this combination of drugs are warranted.

steers and dogs, the half-life ranged from 87 to 164 minutes, respectively. In other studies, the investigators were able to detect YOH in plasma samples at 12 hours postdrug administration and have demonstrated that plasma versus time concentration data were best described by a two-compartment open model [2,3]. They suggest that it was a result of sequestration and slow release over time.

The clearance and terminal elimination half-life can differ substantially between studies because of the ability to collect and detect YOH in plasma samples. However, renal blood flow may be the limiting factor in the clearance of YOH in the form of metabolite. However, additional studies are necessary to be conclusive. The large volume of distribution coupled with the slower systemic clearance is the reason for the longer terminal elimination half-life [2].

Based on analysis of plasma and urine samples in horses, it has been reported that hydroxylation also appears to be the predominant pathway for elimination of YOH. One metabolite identified was hydroxy-yohimbine in urine samples [2]. Yohimbine is eliminated by a first-order process, and it is possible that this could have a very prolonged half-life with small serum concentrations due to release of the drug from tissue reservoirs [2]. A more sensitive YOH assay and determinations of renal and hepatic clearance are necessary to define further a possible prolonged elimination of YOH [9].

Yohimbine has been shown to decrease the incidence of naturally occurring nonpathologic AV conduction disturbances following IV administration in horses [5,18]. Similar effects were reported when administered alone, with maximal resolution of AV blocks occurring within 2 minutes of administration [17].

The clearance and $t_{1/2el}$ (terminal elimination half-life) of YOH following SL DET administration (22.9 mL minute^{-1} kg^{-1}); 1.87 hours ($t_{1/2el}$) reported on a study [6] differs from previous report of YOH disposition following IV DET administration (6.8 mL minute^{-1} kg^{-1}); 4.4 hours ($t_{1/2el}$) [3]. Knych and Stanley (2014) reported total YOH plasma clearance ranged from 18.6 to 41.2 mL minute^{-1} kg^{-1}, indicating that it is a high hepatic extraction ratio drug with extra hepatic metabolism. Similar findings were reported in horses [11,17].

Detomidine IV administration in horses produced decreased heart rate of 15 bpm. This maximal change was observed at 2 minutes postdrug administration, and it is likely attributable to large concentrations of drug delivered to the heart. The heart rate increased 16 bpm in horses receiving YOH subsequent to DET [17]. This change was slightly more rapid, 2 minutes post-YOH administration than that observed when YOH was administered alone. Antagonism of the DET-induced cardiac effects was most pronounced with YOH and tolazoline and least with atipamezole [6]. In addition to their effects on heart rate, administration of DET has been associated with AV conductions blocks increasing incidence of AV blocks [17] following enteral administration. It was reported 48% of the AV signals were blocked following DET administration alone, with the maximal number of conduction blocks occurring by 5 minutes post-DET administration. The bradycardia and the conduction disturbances may be due to a centrally mediated decrease in peripheral sympathetic

Table 3 (continued)

Authors	Objective	Experimental Model	Dose and Administration Route	Pharmacokinetics, Pharmacodynamics, or Behavioral Changes	Conclusion
Knych and Stanley 2014	Describe the effects of alpha-2 adrenergic receptor antagonists on the pharmacodynamics of SL DET in the horse and evaluate effects of alpha-2 adrenergic receptor antagonists in reversing its sedative and cardiovascular effects.	Nine healthy horses consisting of eight Thoroughbreds and one Quarter Horse were studied. Four treatment groups were studied, and each horse received all treatments with a minimum of 1 wk between treatments. Blood samples were obtained and plasma analyzed for YOH, atipamezole, and tolazoline concentrations by liquid chromatography–mass spectrometry. Samples were processed according to the methodology described by (Knych and Stanley 2014).	Four treatments were administered (1) 0.04 mg kg^{-1} DET (Dormosedan Gel, Pfizer Animal Health, NY) SL. (2) 0.04 mg kg^{-1} DET SL followed 1 h later by 0.075 mg kg^{-1} YOH (Yobine, Lloyd Laboratories, IA) IV. (3) 0.04 mg kg^{-1} DET SL followed 1 h later by 4 mg kg^{-1} tolazoline (Tolazine, Lloyd Laboratories) IV. (4) 0.04 mg kg^{-1} DET SL followed 1 h later by 0.12 mg kg^{-1} atipamezole (Antisedan, Pfizer Animal Health) IV.	DET administration significantly increased the prevalence of AV conduction disturbances. Yohimbine effectively decreased the prevalence of the AV blocks initially, although the number of AV blocks increased again by 1–2 h for all drugs. DET significantly increased glucose concentrations by 45 min postadministration with concentrations remaining elevated for 3 h (121 ± 11 and 127 ± 43 mg dL^{-1}). Yohimbine significantly attenuated the DET-induced hyperglycemia.	At the doses of 0.075 mg kg^{-1}, the effects of YOH on cardiac and behavioral effects elicited by SL administration of DET are transient and incomplete.

Abbreviations: AV, atrioventricular; DET, detomidine; IV, intravenous; SL, sublingual; YOH, yohimbine.

tone, presynaptic inhibition of norepinephrine release from fibers innervating the heart, or enhancement of vagal reflexes. Dimaio Knych et al (2012) noted that DET-induced conduction blocks immediately post-YOH administration [3]. The percentage of AV conduction disturbances returned to pre-DET values within 2 minutes of YOH administration.

DET administration following subsequent administration of YOH generates differences in behavior, including a return toward baseline chin-to-ground distances, which were observed within 3 to 5 minutes of YOH administration. This initial period of arousal was followed by 10 minutes of sedation. Although the animals were obviously sedate, signs were less pronounced than those observed upon initial administration of DET [17]. Horses treated with YOH showed signs of alertness within 5 minutes followed by a return to sedation [6].

Plasma glucose concentrations increased following DET administration which decreased toward baseline much faster in groups that received YOH subsequent to DET as compared to horses that did not receive the alpha-2 adrenergic receptor antagonists. It is possible that the faster return to baseline glucose concentrations was due to displacement of DET from receptors. This is supported by the lack of effect of YOH on plasma glucose concentrations [11], suggesting that YOH by itself had no effect on plasma glucose concentrations [17]. Probably, the inhibition of insulin release was mediated through postsynaptic adrenoreceptors located on the pancreatic cells, specifically the alpha-2 adrenergic subtype and that of alpha-2 adrenergic receptor antagonists, such as YOH blocking the hyperglycemic effect of alpha-2 adrenergic agonists [6,19]. Yohimbine decreased DET-induced hyperglycemia due likely to cessation of DET-induced effects as opposed to being due to atipamezole. The antihyperglycemic effect may be dose dependent, and a higher dose of atipamezole may be necessary to reverse the alpha-2 agonistic effect and in turn result in decreased plasma glucose concentrations [6].

DET pronounced generating cardiac effects, including a notable decrease in heart rate and an increased incidence of AV conduction blocks. Conversely, the behavioral effects of the alpha-2 receptor antagonist, YOH, appear to be highly variable between horses. Regardless of the variability in response when administered alone, YOH was effective in resolving naturally occurring as well as DET-induced AV conduction disturbances. Overall, YOH was effective in reversing the behavioral and cardiovascular effects of IV administered DET. The antagonistic effects of YOH on HR and rhythm changes and behavioral effects elicited by SL-administered DET appear to be incomplete.

5. Conclusions

In recent years, DET administration in horses has become popular, in part, due to the existence of a pharmaceutic form supplied as a gel for the oral administration coupled with a YOH administration increment to reverse the DET effects. Although YOH is not an FDA-approved drug for the equines and it is indicated as an alpha-2 antagonist, YOH has been used for this purpose. Scientific evidence shows multiple variation of pharmacodynamics,

pharmacokinetic, and cardiovascular parameters for individual horses; therefore, YOH should be used cautiously in order to avoid serious and unpredictable undesired side effects. Until the causes of variation between individual horses is resolved, it is important to determine if the source of variation relays just in the individuality of each animal or if there is a relationship with determinant factors such as age, sex, body fat or physical condition, renal clearance, or any other condition.

References

[1] Jochle W, Hamm D. Sedation and analgesia with Domosedan (detomidine hydrochloride) in horses: dose response studies on efficacy and its duration. Acta Vet Scand Suppl 1986;82:69–84.
[2] Salonen JS, Vaha-Vahe T, Vainio O, Vakkuri O. Single-dose pharmacokinetics of detomidine in the horse and cow. J Vet Pharmacol Ther 1989;12:65–72.
[3] Knych HK, Steffey EP, Stanley SD. The effects of yohimbine on the pharmacokinetic parameters of detomidine in the horse. Vet Anaesth Analg 2012;39:221–9.
[4] Kaukinen H, Aspegren J, Hyyppa S, Tamm L, Salonen JS. Bioavailability of detomidine administered sublingually to horses as an oromucosal gel. J Vet Pharmacol Ther 2011;34:76–81.
[5] Dimaio Knych HK, Steffey EP, Stanley SD. Pharmacokinetics and pharmacodynamics of three intravenous doses of yohimbine in the horse. J Vet Pharmacol Ther 2011;34:359–66.
[6] Knych HK, Stanley SD. Effects of three antagonists on selected pharmacodynamic effects of sublingually administered detomidine in the horse. Vet Anaesth Analg 2014;41:36–47.
[7] Vainionpaa MH, Raekallio MR, Pakkanen SA, Ranta-Panula V, Rinne VM, Scheinin M, et al. Plasma drug concentrations and clinical effects of a peripheral alpha-2-adrenoceptor antagonist, MK-467, in horses sedated with detomidine. Vet Anaesth Analg 2013;40:257–64.
[8] Grimsrud KN, Mama KR, Thomasy SM, Stanley SD. Pharmacokinetics of detomidine and its metabolites following intravenous and intramuscular administration in horses. Equine Vet J 2009;41:361–5.
[9] Hubbell JA, Sams RA, Schmall LM, Robertson JT, Hinchcliff KW, Muir WW. Pharmacokinetics of detomidine administered to horses at rest and after maximal exercise. Equine Vet J 2009;41:419–22.
[10] Mama KR, Grimsrud K, Snell T, Stanley S. Plasma concentrations, behavioural and physiological effects following intravenous and intramuscular detomidine in horses. Equine Vet J 2009;41:772–7.
[11] Dimaio Knych HK, Stanley SD. Pharmacokinetics and pharmacodynamics of detomidine following sublingual administration to horses. Am J Vet Res 2011;72:1378–85.
[12] Kollias-Baker CA, Court MH, Williams LL. Influence of yohimbine and tolazoline on the cardiovascular, respiratory, and sedative effects of xylazine in the horse. J Vet Pharmacol Ther 1993;16:350–8.
[13] Ramseyer B, Schmucker N, Schatzmann U, Busato A, Moens Y. Antagonism of detomidine sedation with atipamezole in horses. Vet Anaesth Analg 1998;25:47–51.
[14] Hubbell J, Muir W. Antagonism of detomidine sedation in the horse using intravenous tolazoline or atipamezole. Equine Vet J 2006;38:238–41.
[15] Le Corre P, Dollo G, Chevanne F, Le Verge R. Biopharmaceutics and metabolism of yohimbine in humans. Eur J Pharm Sci 1999;9:79–84.

[16] Kamerling SG, Cravens WM, Bagwell CA. Objective assessment of detomidine-induced analgesia and sedation in the horse. Eur J Pharmacol 1988;151:1–8.

[17] DiMaio Knych HK, Covarrubias V, Steffey EP. Effect of yohimbine on detomidine induced changes in behavior, cardiac and blood parameters in the horse. Vet Anaesth Analg 2012;39:574–83.

[18] Dimaio Knych HK, Steffey EP, Deuel JL, Shepard RA, Stanley SD. Pharmacokinetics of yohimbine following intravenous administration to horses. J Vet Pharmacol Ther 2011;34:58–63.

[19] Oda S, Fujimura H, Sasaki Y, Ohneda A. Alpha 2-adrenergic modulation of glucagon and insulin secretions in sheep. Tohoku J Exp Med 1991;163:101–10.