Pharmacodynamics and pharmacokinetics of nalbuphine in xylazine-sedated horses

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Horses, Nalbuphine, Pharmacodynamics, Pharmacokinetics, Xylazine.

Summary
This study describes the selected pharmacodynamics and pharmacokinetics of nalbuphine (NAL) in xylazine (XYL)-sedated horses. Five adult healthy horses were randomly received 2 treatments at a 1-week interval; XYL treatment (0.55 mg/kg IV) and XYL/NAL treatment (XYL, 0.55 mg/kg IV; NAL, 0.3 mg/kg IV). The measured pharmacodynamic variables were sedative and analgesic effects and the effect on ataxia and some physiological parameters. for the pharmacokinetics of NAL, its plasma concentrations were measured using HPLC and a 2-compartment analysis was performed. Greater and prolonged sedation was evident after XYL/NAL treatment compared with XYL treatment. Slightly improved and prolonged analgesia was demonstrated after XYL/NAL treatment. Significant changes in blood pressure and respiratory rate lasted for a shorter duration with XYL/NAL treatment than with XYL treatment. After XYL treatment, rectal temperature was significantly different from baseline and XYL/NAL treatment. Elimination half-life of NAL was 3.47 ± 1.39 hours and total body clearance was 2.88 ± 0.73 L/kg/hour. In conclusion, addition of NAL to XYL resulted in remarkable advantages on the measured parameters. The obtained pharmacokinetics of NAL could be useful in determining the effective NAL infusion rate, which could be further evaluated as an adjunctive agent to XYL for prolonged sedation in horses.

Introduction

The sedative and analgesic effects of α_2-agonists facilitate various diagnostic and surgical procedures in standing horses (Rezende *et al.* 2015). Xylazine (XYL) is a commonly used α_2-agonist in horses. However, it only confers a short duration of sedation and analgesia (Queiroz-Neto *et al.* 1998, Bueno *et al.* 1999). Because sedated horses may respond unexpectedly to imposed stimuli, which can be risky to both horses and practitioners, the administration of XYL or other α_2-agonists alone was also reported to produce less reliable sedation (Alitalo 1986, England *et al.* 1992, Corletto *et al.* 2005). Consequently, these agents can be combined with opioids to enhance and prolong the sedative and the analgesic effects of α_2-agonists (Clarke *et al.*1991, Seo *et al.* 2011, Gozalo-Marcilla *et al.* 2017).

Nalbuphine (NAL) is a semisynthetic agonist-antagonist opioid analgesic with agonistic activity at κ-receptors and antagonistic action at μ-receptors (Kulkarni *et al.* 2015). The NAL-associated analgesia was previously evaluated when it was given in combination with XYL to horses (Brunson and Majors 1987). In another study (Taylor *et al.* 1990), the sedative and cardiorespiratory effects of detomidine/NAL and acepromazine/NAL combinations were also assessed. However, NAL seems to be insufficiently studied in horses.

Considering limited studies targeting the assessment of NAL in horses and the lack of data regarding its pharmacokinetics along with the previously mentioned merits of α_2-agonists/opioids combinations over α_2-agonists alone in terms of concomitant sedation and analgesia, this study aimed to describe the selected pharmacodynamics (involving sedative and analgesic effects and the effect on ataxia and some physiological parameters) and pharmacokinetics of NAL in XYL-sedated horses. We hypothesized that the addition of NAL to XYL might produce better sedative and analgesic effects than XYL alone, without adversely affecting physiological parameters. The used pharmacokinetic model was also hypothesized to be efficient in providing NAL pharmacokinetics.

Materials and methods

Animals

Five adult research horses (4 stallions and 1 mare) of mixed breeds, aged 14.00 ± 4.95 years and weighing 299.80 ± 16.87 kg, were enrolled in this study. The horses were considered healthy based on physical examination, complete blood count, and serum biochemistry analyses. This study was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Sadat City, Egypt. During the study period, horses were kept in their stall at the University of Sadat City where they were fed hay and allowed free access to water.

Study design

This was a randomized, blinded, and crossover study. At the end of the day before the experiment, horses were weighed and the hair over the right and left jugular veins was shaved for placement of intravenous (IV) catheters. Food but not water was withheld for 12 hours before the experiment. At the day of the study, horses were kept in a stock in a quiet room and allowed at least 2 hours to acclimatize to their surroundings. To decrease external stimulation, a fly repellent was also applied to the skin of the horses. The right and left jugular veins were catheterized (using a 16-gauge catheter) under local anesthesia with subcutaneous lidocaine 2% (2 mL) for the administration of drugs and collection of blood samples (in case of XYL/NAL treatment), respectively. Horses were randomly subjected to 2 treatments with 1-week washout period in between. Assigned treatments were as follows:

- XYL treatment: horses received an IV dose of 0.55 mg/kg of XYL (Xyla-Ject 20 mg/mL, Adwia Co., 10th of Ramadan City, Egypt) alone over 1 minute (Fernandes de Souza *et al.* 2012).
- XYL/NAL treatment: horses received an IV dose of 0.55 mg/kg of XYL over 1 minute followed 5 minutes later by an IV dose of 0.3 mg/kg of NAL (Nalufin 20 mg/mL, Amoun Pharmaceutical Co., Cairo, Egypt) (Taylor *et al.* 1990). NAL was administered slowly over 2 minutes.

Assessments

During the study period, sedative and analgesic effects, ataxia, and physiological parameters such as heart rate (HR), respiratory rate (RR), blood pressure, and rectal temperature (RT) were evaluated before (baseline) and at 8, 15, 30, 45, 60, 75, 90, 105, and 120 minutes after XYL administration.

Intestinal motility was also assessed at baseline and at 8, 15, 30, 60, 90, and 120 minutes after XYL administration.

Evaluation of sedative effect (degree and duration of sedation)

A multifactorial sedation scale (MFSS) was designed to evaluate the degree of sedation and involved a summation of the scores of head drop percentage and the scores of horse response to auditory, visual, and tactile stimuli (Table I).

The head drop percentage was estimated by measuring the change in head height (distance from the most ventral bony portion of the chin to the ground in centimeters) with respect to the baseline head height using a measuring tape attached to the chin region (Solano *et al.* 2009, Rezende *et al.* 2014). To determine the baseline head height, the horses were observed for 15 minutes before drug administration, and the most frequently observed head position was recorded as the baseline head height. A numerical rating scale (NRS) modified from Solano and colleagues (Solano *et al.* 2009) was used to score the head drop percentage.

For auditory stimulation of the studied horses, hand clapping was conducted 60 centimeters from the horses' head (Love *et al.* 2011), and their response was scored using a scale modified from Wagner and colleagues (Wagner *et al.* 2011). Visual stimulation was done by waving a piece of cloth toward the horses' head (Peştean *et al.* 2010) with scoring of their reaction using a scale adopted from Ringer and colleagues (Ringer *et al.* 2013). Tactile stimulation was done by touching inside the pinna of the ear using a pen (Gozalo-Marcilla *et al.* 2017) with subsequent scoring for the resultant response using a scale modified from Clarke and colleagues (Clarke *et al.* 1991).

The duration of sedation was considered as the period over which significant changes in sedation scores were determined.

Evaluation of analgesic effect (degree and duration of analgesia)

The degree of analgesia was assessed using 2 nociceptive stimuli, namely, needle prick and electrical stimulation. For the needle prick, a 22-gauge, 1.25-inch needle was thrusted to its whole length just once at the right and left flank regions, alternatively. The horses' response to this stimulus was graded using the NRS adapted from Wagner and colleagues (Wagner *et al.* 2011) (Table II).

For electrical stimulation, an electrical stimulus (100 Hz and 1 ms) was applied to the horses' skin at the

Table I. *The multifactorial sedation scale (MFSS) used to score degree of sedation (scores of head drop% and the horse response to auditory, visual, and tactile stimuli).*

Variable	Score	Description
Head drop %	0	No head lowering (head height was equal or higher than baseline)
	1	Head slightly lowered (<40%)
	2	Head moderately lowered (40–60%)
	3	Head markedly lowered >60%
Response to auditory stimulation	0	Horse raised its head briskly and ears were erected or laid back
	1	Horse calmly raised its head
	2	Ear twitched or horse moved slightly
	3	No observable response
Response to visual stimulation	0	Undiminished response, animal move away vigorously
	1	Muted response or subdued reaction and movements
	2	Reaction significantly subdued (elevates head slightly)
	3	No signs of visual arousal.
Response to tactile stimulation	0	Rapid and marked response (head shake) to stimulation. It was also awarded when test was extremely difficult to be performed.
	1	The response was easily elucidated but was slower.
	2	The response was sluggish and only elicited by prolonged stimulation.
	3	No response.
Total score of sedation degree	0-3	No sedation
	4-6	Mild sedation
	7-9	Moderate sedation
	10-12	Intense sedation

shoulder region using 2 adhesive electrodes of an electric muscle stimulator device (Healthtronic muscle stimulator JH4207, China). The electrodes were placed 7 cm apart and secured in position using a sheet of adhesive and transparent medical tape. The intensity of electrical stimulus was gradually increased until the animal turned its head back toward the stimulus (avoidance response), and this intensity was considered the electrical nociceptive threshold.

The electrodes of the unit were connected to an oscilloscope (Fluke PM3394A, Science Park Eindhoven 5110, Son, 5692 EC Netherlands) to determine the exact voltage of the recorded electrical stimuli (electrical nociceptive thresholds) throughout the study.

The duration of analgesia was the period over which there were significant changes in electrical nociceptive threshold and the scores of animal response to the needle prick.

Table II. *Numerical rating scale (NRS) used to assess analgesic effect in needle prick method.*

NRS	Response to pinprick
1	Dramatic response (the horse was alert and moved, kicked, or bit)
2	Moderate response (the horse raised its head briskly and ears were erect or laid back)
3	Mild response (the horse calmly raised its head)
4	Slight or barely perceptible response (the ear twitched or horse moved slightly)
5	No observable response

Evaluation of ataxia

The degree of ataxia was assessed using the NRS described by Fernandes de Souza and colleagues (Fernandes de Souza *et al.* 2012) (Table III).

Throughout the study period, a single blinded investigator evaluated sedation, analgesia, and ataxia to enhance the reliability of the adopted assessment scales. Over the entire observation period, the horses were also monitored for any central nervous system excitatory signs such as muscle tremors.

Evaluation of physiological parameters

Intestinal motility, HR, RR, blood pressure, and RT

Table III. *Numerical rating scale used to assess degree of ataxia.*

NRS	Degree of ataxia
0	No ataxia.
1	Mild ataxia. The horse was stable but slightly swaying
2	Moderate ataxia. The horse was swaying and leaning against the stock
3	Intense ataxia. The horse was leaning against the stock and swaying with its hind limbs crossed and its forelimbs buckling at the carpal joints

were evaluated. For intestinal motility, each of the right upper, right lower, left upper, and left lower abdominal quadrants were auscultated for 30 seconds and intestinal sounds were scored according to Dhanjal and colleagues (Dhanjal *et al.* 2009) in which > 2 sounds in 30 seconds scored 2, 1-2 sounds scored 1, and no sounds scored 0. Therefore, the cumulative score from all 4 quadrants ranged from 0 to 8. HR was measured by auscultation with a stethoscope for over 1 minute. RR was monitored by counting the number of thoracic excursions for over 1 minute.

Noninvasive measurement of arterial pressures [systolic (SAP), diastolic (DAP), and mean (MAP)] was performed via an oscillometric technique (Contec patient monitor, Contec Medical Systems Co., Qinhuangdao, China) with the cuff positioned at the tail base (adult noninvasive blood pressure cuff, 19-24 cm). The recorded blood pressure values were further corrected according to the height difference between the cuff and shoulder joint (1 cm H_2O = 0.7355 mmHg) with the latter location representing the level of the right atrium of the heart (Gozalo-Marcilla *et al.* 2017). RT was measured in degrees Celsius (°C) using a digital thermometer.

Pharmacokinetic study of nalbuphine

Sample collection

During the evaluation of XYL/NAL treatment, venous blood samples (10 mL) were collected at baseline and at 1, 3, 8, 15, 23, 38, 53, 68, 83, 98, 113, 240, 360, 480, and 600 minutes after NAL administration. Before the collection of the 10-mL blood sample, 2 mL of blood was withdrawn and discarded. The blood samples were transferred immediately into heparinized tubes and centrifuged for 10 minutes at 2,400 g (tabletop centrifuge, Gemmy Industrial Corp, Min Chuan West Road, Taipei, Taiwan). Plasma was harvested and stored at - 80 °C until assayed.

Determination of plasma nalbuphine concentration

The concentrations of NAL in equine plasma were quantitated by high-performance liquid chromatography analysis with an ultraviolet detection. Stock solution was prepared by dissolving 10 mg of NAL hydrochloride in 10-mL water. Working solutions were prepared by serially diluting NAL stock solution. Plasma calibrators were prepared by diluting working solutions with drug-free equine plasma to concentrations of 1 (the limit of quantitation), 5, 10, 25, 50, 100, 250, 500, 1,000, and 2,500 ng/mL.

Extraction of NAL from plasma samples was performed using the method previously published by Huang and colleagues (Huang *et al.* 2013) using the Agilent 1200 Series liquid chromatography system (Agilent community, Santa Clara, United States).

Pharmacokinetic analysis

Plasma concentrations of NAL versus time curve were generated and best fitted by 2-compartment open model system with the aid of computer polyexponential curve stripping program (RSTRIP, Micromath Scientific Software, Salt Lake City, UT, USA). Data from each horse were fitted individually, and the pharmacokinetic variables were computed using the software programs. The hybrid rate constants of the distribution and elimination phases (α and β) and elimination rate constant (K_{el}) and corresponding extrapolated zero-time intercepts of distribution and elimination phases (A and B) were calculated. Distribution and elimination half-lives ($t_{0.5\alpha}$, $t_{0.5\beta}$), and K_{12} and K_{21} (distribution rate constants from central to peripheral and from peripheral to central compartment, respectively) were also calculated. The area under the curve from zero to infinite time ($AUC_{0-\infty}$) and mean residence time were calculated. Other pharmacokinetic parameters such as total body clearance (Cl_{Tot}), the volume of the central compartment (V_c), apparent volume of distribution ($V_{d\ area}$), volume of distribution in terminal elimination phase ($V_{d\beta}$), and the volume of distribution at steady state (V_{dss}) were calculated by standard methods (Baggot 1978, Dhanjal *et al.* 2009).

Statistical analysis

Statistical analysis was performed with SPSS 16.0 software (SPSS, USA). The parametric variables such as the head drop percentage, electrical nociceptive threshold, and physiological parameters (HR, RR, SAP, DAP, MAP, and RT) were analyzed using a one-way analysis of variance (ANOVA) with Dunnett's post-test for comparisons of means within each group in relation to baseline. Comparisons between groups at each time were performed with one-way ANOVA followed by Tukey's test. The Friedman test was used for the nonparametric variables such as the response to needle prick and auditory, visual, and tactile stimuli and sedation, ataxia, and intestinal motility. The results of different parametric variables were expressed as mean ± standard deviation with exception of head drop percentage and plasma concentration of nalbuphine which were expressed as mean. The results of non-parametric variables were expressed as median (range), and $p < 0.05$ was considered statistically significant.

Results

A significant head drop was evident after both treatments compared with baseline (30 minutes with XYL and 45 minutes with XYL/NAL). The deepest drop (59.65%) was recorded at 8 minutes with XYL, whereas the greatest drop (71.21%) was evident at 15 minutes with XYL/NAL. The head was significantly dropped with XYL/NAL compared with XYL at 15 and 30 minutes (Figure 1).

The horses' reaction to auditory and tactile stimuli was significantly reduced from baseline at 8 minutes with XYL and at 8 and 15 minutes with XYL/NAL. The horses' response to both stimuli was significantly lower with XYL/NAL than with XYL at 15 minutes. The horses' response to visual stimulation was significantly reduced from baseline at 8 and 15 minutes with both treatments without a significant difference in between (Table IV).

The highest degree of sedation (based on sedation scores) with XYL was evident at 8 minutes, which was evidenced by moderate sedation in all studied horses (5 of 5), whereas sedation with XYL/NAL was highest at 15 minutes, which was evidenced by moderate to intense sedation in the treated horses (3 of 5 and 2 of 5, respectively). In addition, at 15 minutes, sedation was significantly greater with XYL/NAL than with XYL. Concerning the duration of sedation (significant increase in sedation scores), sedation persisted for 15 and 45 minutes with XYL and XYL/NAL, respectively (Table IV).

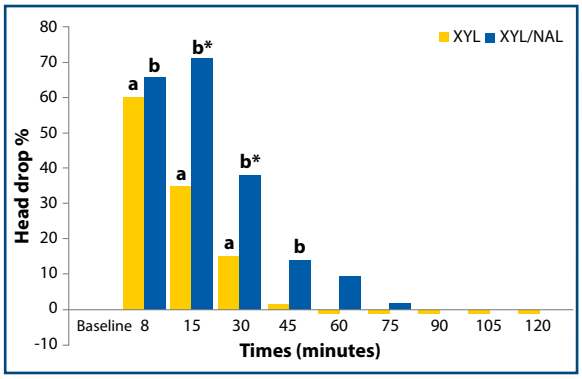

Figure 1. *The mean values of head drop % in horses after receiving xylazine (XYL) and xylazine/nalbuphine (XYL/NAL) treatments.*
[a,b]Significant difference ($p<0.05$) from the respective baseline value for XYL[a] and XYL/NAL[b]. *Significant difference ($p<0.05$) from the value of XYL tratment.

Analgesia (for needle prick and electrical stimulation) persisted for 8 minutes with XYL and 15 minutes with XYL/NAL. Occasionally, analgesia was slightly better (not significant) with XYL/NAL than with XYL (Table V).

Ataxia scores were significantly higher than baseline only at 8 minutes with XYL and from 8 to 30 minutes with XYL/NAL. Ataxia was significantly higher with XYL/NAL than with XYL at 15 and 30 minutes; however, moderate ataxia was the highest degree exhibited by the studied horses (1 of 5 with XYL and 3 of 5 with XYL/NAL) after either treatment (Table V).

A significant difference in intestinal motility scores and HR was not detected from baseline with both treatments or in between at any observation time (Tables V and VI). A significant decrease in SAP, DAP, MAP, and RR was detected for a shorter duration with XYL/NAL than with XYL compared with baseline without being significantly different among treatments (Table VI). A significant decrease in RT from baseline was only detected with XYL treatment at 8 and 15 minutes with being significantly lower with XYL than XYL/NAL at 8 minutes only (Table VI).

The observation of the horses throughout the study period revealed an occurrence of muzzle tremors in 1 horse with XYL and 2 horses with XYL/NAL.

Regarding the pharmacokinetic study of NAL, a peak plasma concentration of 79.64 ng/mL was attained at 8 minutes after NAL administration. After 8 minutes, NAL concentration declined gradually over time, and its level was below the limit of quantification

Table IV. *Median (range) of response scores to auditory, visual, and tactile stimuli and sedation scores in horses after receiving xylazine (XYL) and xylazine/nalbuphine (XYL/NAL) treatments.*

Variable	Treatment	Baseline	Time (minutes)								
			8	15	30	45	60	75	90	105	120
Response scores to auditory stimulation	XYL	1 (1-2)	2 (2-3)*	1 (1-2)	1 (1-2)	1 (1-2)	1 (1-2)	1 (1-2)	1 (1-2)	1 (1-2)	1 (1-2)
	XYL/NAL	1 (1-2)	2 (2-3)*	3 (2-3)*‡	2 (1-3)	1 (1-2)	1 (1-2)	1 (1-2)	1 (1-2)	1 (1-2)	1 (0-2)
Response scores to visual stimulation	XYL	0 (0-1)	1 (1-2)*	1 (1-1)*	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
	XYL/NAL	0 (0-1)	1 (1-3)*	1 (1-3)*	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
Response scores totactile	XYL	0 (0-1)	1 (1-2)*	1 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
	XYL/NAL	0 (0-1)	2 (1-2)*	2 (1-2)*‡	1 (0-1)	1 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
Sedation scores	XYL	2 (1-3)	7 (7-8)*	5 (4-5)*	3 (1-4)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)
	XYL/NAL	2 (1-3)	8 (7-11)*	8 (7-11)*‡	4 (3-5)*	3 (2-4)*	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)

*Significant difference ($p < 0.05$) from the respective baseline value; ‡Significant difference ($p < 0.05$) from the value of XYL treatment.

in 2 horses (horse number 3 and 5) at 600 minutes whereas still being detected in others (Figure 2). All pharmacokinetic parameters obtained from the 2-compartment open model system and the used pharmacokinetic equations are presented in Table VII.

Discussion

Head drop is an objective indicator for sedation in horses (Ringer *et al.* 2013), which was previously judged to be satisfactory in assessing the sedative effect of α_2-agonists and α_2-agonists/opioids combinations (Solano *et al.* 2009, Guilhen *et al.* 2015). Accordingly, in this study, it was involved in the designed MFSS. A significant head drop was evidenced after XYL (0.55 mg/kg) administration (XYL treatment) to the studied horses. Consistently, in a previous report (Santonastaso *et al.* 2014), a significant head lowering was demonstrated after an IV administration of 0.6 mg/kg of XYL to horses. Even if, in the current study, XYL produced a shorter duration of head drop than the later

Table V. *The Median (range) of response scores to needle prick, Mean ± SD values of electrical nociceptive threshold and Median (range) of ataxia and intestinal motility scores in horses after receiving xylazine (XYL) and xylazine/nalbuphine (XYL/NAL) treatments.*

Variable	Treatment	Baseline	Time (minutes)								
			8	15	30	45	60	75	90	105	120
Response scores to needle prick	XYL	2 (1-3)	3 (3-4)*	3 (1-4)	2 (1-4)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)
	XYL/NAL	2 (1-3)	3 (3-4)*	3 (3-4)*	3 (2-4)	2 (2-4)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)	2 (1-3)
Electrical nociceptive threshold	XYL	1.92 ± 0.35	2.58 ± 0.47*	2.30 ± 0.27	2.24 ± 0.02	1.92 ± 0.35	1.92 ± 0.35	1.92 ± 0.35	1.92 ± 0.35	1.92 ± 0.35	1.92 ± 0.35
	XYL/NAL	1.85 ± 0.26	2.81 ± 0.39*	2.97 ± 0.53*	2.43 ± 0.36	1.98 ± 0.46	1.85 ± 0.26	1.85 ± 0.26	1.85 ± 0.26	1.85 ± 0.26	1.85 ± 0.26
Ataxia scores	XYL	0 (0-0)	1 (1-2)*	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
	XYL/NAL	0 (0-0)	2 (1-2)*‡	2 (1-2)*‡	1 (0-1)*‡	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Intestinal motility scores	XYL	4 (4-5)	4 (4-4)	4 (4-4)	4 (4-4)	ND	4 (4-4)	ND	4 (4-5)	ND	4 (4-5)
	XYL/NAL	4 (4-6)	4 (3-5)	4 (3-5)	4 (4-5)	ND	4 (4-6)	ND	4 (4-6)	ND	4 (4-6)

*Significant difference ($p < 0.05$) from the respective baseline value; ‡Significant difference ($p < 0.05$) from the value of XYL treatment; ND = Not determined.

Table VI. *Mean ± SD values of heart rate (HR), arterial pressures (systolic (SAP), diastolic (DAP) and mean (MAP)), respiratory rate (RR) and rectal temperature (RT) in horses after receiving xylazine (XYL) and xylazine/nalbuphine (XYL/NAL) treatments.*

Variable	Treatment	Baseline	Time (minutes)								
			8	15	30	45	60	75	90	105	120
HR (beats/minute)	XYL	35.67 ± 4.04	30.67 ± 2.52	31.67 ± 3.06	32.33 ± 3.51	33.33 ± 2.52	33.67 ± 3.06	34.33 ± 3.21	35.00 ± 5.29	35.33 ± 5.86	35.67 ± 4.04
	XYL/NAL	36.67 ± 3.06	35.00 ± 3.61	35.33 ± 3.51	35.67 ± 3.06	35.67 ± 3.06	36.00 ± 3.00	36.67 ± 3.06	36.67 ± 3.06	36.67 ± 3.06	34.00 ± 4.58
SAP (mmHg)	XYL	119.67 ± 6.03	99.00 ± 14.11*	97.33 ± 10.07*	92.00 ± 7.21*	98.67 ± 11.68*	109.00 ± 5.29	114.33 ± 1.53	110.67 ± 9.71	116.33 ± 2.52	117.67 ± 4.73 1
	XYL/NAL	123.33 ± 5.51	111.00 ± 3.61*	108.67 ± 2.08*	105.67 ± 4.93*	116.67 ± 2.08	117.33 ± 2.52	119.00 ± 1.00	120.33 ± 2.52	121.33 ± 3.51	123.00 ± 5.00
DAP (mmHg)	XYL	77.33 ± 2.52	64.67 ± 4.73*	61.67 ± 1.53*	64.00 ± 1.00*	70.67 ± 1.53	71.67 ± 2.52	73.67 ± 4.51	71.33 ± 2.52	74.00 ± 4.58	72.67 ± 4.93
	XYL/NAL	78.00 ± 3.61	65.00 ± 4.58*	63.67 ± 4.04*	65.33 ± 6.11*	71.00 ± 6.25	75.00 ± 7.00	74.33 ± 4.04	75.00 ± 4.58	73.00 ± 7.55	77.00 ± 2.65
MAP (mmHg)	XYL	92.36 ± 2.29	77.05 ± 3.45*	74.44 ± 5.59*	74.57 ± 4.14*	80.84 ± 4.57*	83.33 ± 7.02	88.09 ± 1.28	85.00 ± 5.00	88.47 ± 1.44	87.36 ± 2.04
	XYL/NAL	94.35 ± 2.44	81.85 ± 2.39*	79.68 ± 4.64*	80.63 ± 2.29*	85.96 ± 5.16	92.93 ± 6.95	90.07 ± 1.04	91.47 ± .82	91.98 ± 2.25	92.68 ± 1.25
RR (breaths/minute)	XYL	15.67 ± 1.53	9.00 ± 1.00*	9.33 ± 1.53*	10.33 ± 0.58*	11.00 ± 1.73*	13.67 ± 2.08	14.00 ± 1.73	14.67 ± 1.15	15.00 ± 1.00	15.00 ± 1.00
	XYL/NAL	16.00 ± 1.73	11.67 ± 1.15*	12.33 ± 0.58*	13.00 ± 1.00	15.00 ± 1.00	16.00 ± 1.73	16.00 ± 1.73	16.00 ± 1.73	16.00 ± 1.73	16.00 ± 1.73
RT (°C)	XYL	38.07 ± 0.15	36.83 ± 0.15*‡	36.57 ± 0.55*	37.30 ± 0.30	37.33 ± 0.45	37.20 ± 0.66	37.50 ± 0.40	37.53 ± 0.45	37.63 ± 0.46	37.70 ± 0.52
	XYL/NAL	37.93 ± 0.15	37.60 ± 0.26	37.50 ± 0.40	37.67 ± 0.25	37.80 ± 0.10	37.90 ± 0.10	37.83 ± 0.25	37.80 ± 0.30	37.83 ± 0.23	37.93 ± 0.06

*Significant difference ($p < 0.05$) from the respective baseline value; ‡Significant difference ($p < 0.05$) from the value of XYL treatment.

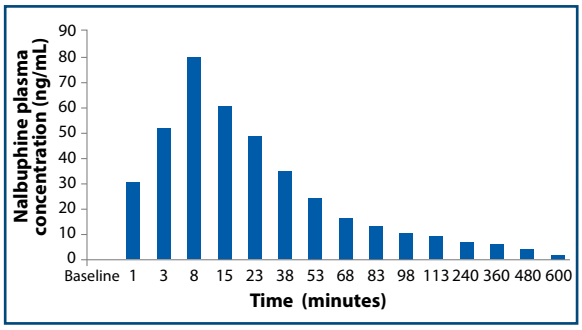

Figure 2. *The mean plasma concentrations of nalbuphine in xylazine premedicated horses.*

study (30 minutes versus 75 minutes) despite of approximately similar doses used in both.

A longer and greater head drop was obtained when NAL was given in combination with XYL (XYL/NAL treatment) to horses, which might have resulted from the ability of NAL to potentiate the sedative effect of XYL or a synergistic action in between. A synergistic sedative effect of opioids and α_2-agonists combinations was similarly reported by other authors (Corletto *et al.* 2005).

For a comprehensive estimation of the sedative effect of the studied treatments, MFSS used in this study included the assessment of both head drop and commonly used variables for sedation quality, that is, the horses' response to auditory, visual, and tactile stimuli (Ringer *et al.* 2013, Schauvliege *et al.* 2019).

The horses' response to auditory, visual, and tactile stimuli was greatly reduced after XYL administration. Similar findings were reported after romifidine administration to horses (Clarke *et al.* 1991). In this study, XYL maintained reduced reaction to the inflicted stimuli for a shorter duration than head drop. Consistent findings were described by Rezende and colleagues (Rezende *et al.* 2015) after dexmedetomidine administration to horses. The addition of NAL to XYL produced a greater depression of horses' response to some stimuli, which agrees with the findings by Clarke and colleagues (Clarke *et al.* 1991) when butorphanol was given along with romifidine to horses.

According to the designed scoring system, XYL (0.55 mg/kg) provided sedation for 15 minutes. In agreement, Hubbell (Hubbell 2009) reported sedation for only 30 minutes after administration of 1 mg/kg of XYL to horses. Therefore, the shorter sedative effect observed in our study could be parallel to a lower tested dose. In a previous study (Taylor *et al.*1990), NAL was efficient in enhancing detomidine-associated sedation in horses, which agrees with our findings. In our study, XYL/NAL administration achieved longer sedation and analgesia than XYL. Similarly, prolonged sedation and analgesia was described in horses treated with romifidine/butorphanol combination compared with those treated with romifidine alone (DeRossi *et al.* 2009).

Administration XYL/NAL was associated with only slightly better analgesia than XYL. However, we could hypothesize that the analgesic effect of this combination would be better expressed in horses subjected to a true clinical pain, which responds better to opioid analgesics, compared with the implemented experimental pain (Clutton 2010).

In this study, XYL was evaluated at a dose of 0.55 mg/kg. This was based on the publication by Fernandes de Souza and colleagues (Fernandes de Souza *et al.* 2012) in which the same dose followed by a constant rate infusion of XYL (1.1 mg/kg/hour) was associated with mild ataxia in most studied horses. Likewise, mild ataxia was the most frequently exhibited ataxia by our horses. This ataxia could be attributed to the muscle relaxant effect of XYL mediated by its binding to α_2-adrenoreceptors in the spinal cord (Sinclair

Table VII. *Pharmacokinetic variables following intravenous administration of nalbuphine (0.3 mg/kg) in xylazine premedicated horses.*

Horse	B (ng/h)	A (ng/h)	β h^{-1}	α h^{-1}	$AUC_{(0\text{-inf})}$ (ng/mL/h)	Variable										
						MRT h	$T_{0.5\beta}$ h	$T_{0.5\alpha}$ h	K_{12} h^{-1}	K_{21} h^{-1}	V^c (L/kg)	$V_{d\beta}$ (L/kg)	V_{dss} (L/kg)	$V_{d\,area}$ (L/kg)	K_{el} h^{-1}	Cl_{Tot} (L/kg/h)
1	14.8	100.79	0.16	3.5	109.76	4.66	4.22	0.19	3.13	0.58	2.605	20.27	16.66	17.08	0.97	2.73
2	23.8	123.96	0.26	5.01	120.63	3.08	2.67	0.14	4.25	1.03	2.03	12.61	10.41	9.57	1.26	2.49
3	11.01	84.87	0.15	3.02	103.17	4.78	4.52	0.23	2.69	0.48	3.13	27.25	20.67	19.39	0.94	2.91
4	17.42	108.03	0.15	3.94	137.91	5.31	4.53	0.18	3.42	0.68	2.39	17.22	14.41	14.50	0.87	2.18
5	21	90.71	0.49	4.04	73.13	1.41	1.40	0.17	3.37	1.16	2.69	14.29	10.50	8.37	1.71	4.10
Mean ± SD	17.61 ± 5.03	101.67 ± 15.33	0.24 ± 0.15	3.90 ± 0.74	108.92 ± 23.94	3.85 ± 1.60	3.47 ± 1.39	0.18 ± 0.03	3.37 ± 0.57	0.79 ± 0.29	2.57 ± 0.40	18.33 ± 0.40	14.53 ± 4.34	13.78 ± 4.74	1.15 ± 0.35	2.88 ± 0.73

B = Extrapolated zero-time intercept associated with the elimination phase; A = Extrapolated zero-time intercept associated with the distribution phase; β = Hybrid rate constant of the elimination phase; α = Hybrid rate constant of the distribution phase; $AUC0_{-\infty}$ = Area under the curve from zero to infinite time; MRT = Mean residence time; $t_{0.5\beta}$ = Elimination half-life; $t_{0.5\alpha}$ = Distribution half-life; K_{12} = Distribution rate constant from central to peripheral compartment; K_{21} = Distribution rate constant from peripheral to central compartment; V_c = The volume of the central compartment; $V_{d\beta}$ = Volume of distribution in terminal elimination phase; V_{dss} = The volume of distribution at steady state; $V_{d\,area}$ = Apparent volume of distribution; K_{el} = Elimination rate constant; Cl_{Tot} = Total body clearance.

2003). Ataxia was aggravated and prolonged when NAL was combined with XYL. Similar data were described by DeRossi and colleagues (DeRossi *et al.* 2009) and Ringer and colleagues (Ringer *et al.* 2012) after the administration of α_2-agonists in combination with butorphanol versus α_2-agonists alone. However, the resultant moderate ataxia was acceptable during various procedures in standing horses (Solano *et al.* 2009, Ruiz *et al.* 2015).

For intestinal motility, NAL seemed to have a significant effect on this parameter, which was elucidated by non-significant difference between XYL/NAL and XYL. Conversely, significantly reduced motility was recorded after the administration of a butorphanol bolus to horses (0.1 mg/kg IV) (Sellon *et al.* 2001). These findings might elucidate the milder effect of NAL on intestinal motility than the commonly used agonist-antagonist opioid, butorphanol.

The addition of NAL to XYL did not exaggerate its depressant effects on SAP, DAP, MAP, and RR and even reduced the duration of these changes. This might be caused by a higher incidence of muscle tremors associated with NAL administration that might result in a slight degree of sympathetic stimulation.

Administration of XYL resulted in significant reduction in RT. On the contrary, a significant increase in RT was reported by other authors (Seo *et al.* 2011) after XYL administration. This discrepancy could be attributed to a higher XYL dose used in their study (Seo *et al.* 2011) than ours, which induced slightly increased peripheral vasoconstriction that might offset body heat loss (Dugdale 2010).

Determining the plasma concentration of NAL revealed that a peak concentration of 79.64 ng/mL was attained at 8 minutes after its administration, which coincided with the 15-minute observation period after XYL administration. This might explain the greater sedation and analgesia detected with XYL/NAL at this time compared with others. Such observation might also propose 79.64 ng/mL to be set as the target concentration of NAL in horses, which seemed to be 4 times higher than that previously reported for morphine in horses (Bugedo and Torregrosa 1995).

The 2-compartment model was previously efficient in describing tramadol disposition after IV administration of 2 mg/kg in horses (Dhanjal *et al.* 2009). Based on this, we used a similar model to conduct the pharmacokinetic analysis of NAL.

According to the used pharmacokinetic model, the elimination half-life for NAL was 3.47 ± 1.39 hours whereas the total body clearance was 2.88 ± 0.73 L/kg/hour. This might indicate higher elimination half-life and lower clearance rate for NAL in horses than those (elimination half-life, 0.68 hour; clearance rate, 6.86 L/kg/hour) demonstrated in calves after the administration of a slightly higher NAL dose by the same route (Coetzee *et al.* 2014). In consistent with our data, NAL presented a comparable elimination half-life (3.7 hour) after IV administration to humans (Aitkenhead *et al.* 1988).

Conclusions

Comparing XYL and XYL/NAL treatments elucidated some of the pharmacodynamic effects of NAL in XYL-sedated horses. From this, the addition of NAL to XYL seemed to have remarkable advantages on the measured pharmacodynamic parameters (improved duration of sedation and analgesia, superior degree of sedation, and better effect on physiological parameters). In addition, ataxia was still acceptable and no considerable adverse events were encountered when NAL was combined with XYL. The pharmacokinetics of NAL also provided information about the most effective concentration of NAL and the clearance rate; both could be used to calculate the effective NAL infusion rate. Such rate could be further evaluated as an adjunctive agent to XYL for prolonged sedation in horses that could extensively express the role of NAL as a sedative adjuvant in horses.

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