Effects of intravenous romifidine, detomidine, detomidine combined with butorphanol, and xylazine on tear production in horses.

EFFECTS OF INTRAVENOUS ROMIFIDINE, DETOMIDINE, DETOMIDINE COMBINED WITH BUTORPHANOL, AND XYLAZINE ON TEAR PRODUCTION IN HORSES

Running title: effects of sedation on equine STT I values

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SUMMARY

Sedation facilitates the ocular examination in horses. Alpha2-adrenoceptor agonists are commonly used in equine practice. If the eye is painful, the combination of an α2-adrenoceptor agonist and butorphanol provides a greater analgesic effect. Unfortunately, little is known about the effects of α2-adrenoceptor agonists on equine Schirmer tear test I (STT I) values. The aim of the study was to assess the effects of intravenous romifidine, detomidine, detomidine combined with butorphanol, and xylazine on the STT I values in horses. Forty client-owned Italian saddle horses were enrolled. Horses received 0.04 mg/kg of romifidine or 15 µg/kg of detomidine or 10 µg/kg of detomidine combined with 10 µg/kg of butorphanol or 0.7 mg/kg of xylazine intravenously. The Schirmer tear test strip was inserted into the lateral third side of the inferior conjunctival fornix for one minute in each eye. The STT I measurements were performed before sedation and at 5, 15, 30, 60, 120 and 180 minutes after the administration of sedation. The data were analyzed by ANOVA. Romifidine did not affect the STT I values. Detomidine significantly reduced the STT I values at 15 minutes (18.17 ± 0.97 mm/min). The combination of detomidine and butorphanol significantly reduced the STT I values at 30 and 60 minutes (17.44 ± 0.99, 15.81 ± 0.99 mm/min). Xylazine significantly increased the STT I values at 5, 15 and 30 minutes (25.17 ± 0.99, 26.72 ± 0.99, 28.07 ± 0.99 mm/min). The STT I values at 180 minutes were similar to those before sedation. These results suggest that the administration of xylazine or detomidine alone or combined with butorphanol is associated with significant changes in aqueous tear production, whereas romifidine does not affect the STT I values. Romifidine is therefore suitable for chemical restraint to measure tear production in horses.

Key words: horse, Schirmer tear test I, sedation, α2-adrenoceptor agonist, butorphanol

INTRODUCTION

The tear film confers the cornea an optical surface for the refraction of light, allows the mechanical removal of debris and bacteria, and lubricates the conjunctiva (Martin 2005). If a deficiency of the lacrimal system is supposed, the measurement of tear production is mandatory (Crispin 2000). The Schirmer tear test I (STT I) is the most commonly used test to measure the basal and reflex tear production in horses (Williams *et al.* 1979). Low STT I values than 10 mm/min are pathological, whereas high values are not pathological because horse's tear production may be as high as 35 mm/min (Hendrix 2005).

A single intravenous standard dose of an α2-adrenoceptor agonist is commonly satisfactory to perform a complete ocular examination in horses (Hendrix 2005). If the eye is painful, the combination of an α2-adrenoceptor agonist and butorphanol may be required to provide a greater analgesic effect (Hendrix 2005; Muir 2009; Bianchi *et al.* 2015).

Alpha2-adrenoceptor agonists induce a dose-dependent, rapid and quite predictable effect (Muir 2009). Romifidine, detomidine and xylazine are the most commonly used α2-adrenoceptor agonists in equine practice (Nannarone *et al.* 2007). They vary in potency, duration of action, and side effects depending on α2-adrenoceptor selectivity. Romifidine is more potent than xylazine but it is less potent than detomidine (Muir 2009). Nevertheless, 0.04 mg/kg of romifidine appeared equipotent to 10 µg/kg of detomidine, whereas 0.08 mg/kg of romifidine appeared similar in potency to 1 mg/kg of xylazine (England *et al.* 1992).

The duration of action of detomidine is longer compared to that of xylazine but shorter compared to that of romifidine (Muir 2009).

With regard to side effects, the depression of cardiorespiratory function induced by xylazine and romifidine is less marked than that caused by detomidine. Furthermore, romifidine and detomidine induce less pronounced ataxia compared to that induced by xylazine (Muir 2009).

Butorphanol is a κ-opioid receptor agonist and a μ-opioid receptor antagonist. It is not commonly used alone for an ocular examination because it may cause increased spontaneous movements. The combination of butorphanol and α2-adrenoceptor agonist synergistically acts to improve sedative effects (Muir 2009).

Conflicting data about the effects of α2-adrenoceptor agonists on equine STT I values have been previously reported. Intravenous administration of 20 μg/kg of detomidine or 150 mg of xylazine significantly reduced the STT I values (Brightman *et al.* 1983; Ghaffari *et al.* 2017). On the contrary, Marts *et al.* (1977) reported that xylazine had no demonstrable effect on tear production in horses. To the authors' knowledge, there are no available data on the effects of romifidine and detomidine combined with butorphanol on equine tear production.

The aims of the study were to evaluate the effects of intravenous romifidine, detomidine, detomidine combined with butorphanol, and xylazine on tear production as assessed with the STT I in horses and to evaluate the influence of the sex and right eye or left eye on the STT I values after sedation.

MATERIALS AND METHODS

Animals

This study was performed in accordance with the Legislative Decree n. 26 of 4th March 2014 under Italian Animal Welfare Legislation and was approved by the Institutional Ethics Committee for animal welfare of the University of Parma. The owners signed a voluntary informed consent form prior to the horses' enrollment.

The inclusion criteria were as follow: adult Italian saddle horses undergoing a radiologic or ultrasonographic assessment, no history of previous ocular disease, no drug therapy in the previous two months, and normal complete blood count and biochemical profile.

The exclusion criteria were as follow: horses with abnormalities of the ocular surface or with STT I values lower than 15 mm/min.

Schirmer tear test I measurements

An ocular examination consisting of a Schirmer tear test[1] and a slit lamp examination (SL-17[2]) was performed before sedation. The strip was inserted into the lateral third of the inferior conjunctival fornix for one minute in each eye. The STT I was performed first in the right eye.

The STT I readings were performed before sedation, at 5, 15, 30, 60, 120 and 180 minutes after the administration of sedation. The STT I measurements were performed in an enclosed space under the same conditions of light, temperature and relative humidity. The same experienced veterinarian blinded from the treatment performed the STT I measurements.

Sedation protocols

Food but not water was withheld for 12 hours prior to the administration of the sedation. Horses were randomly (simple randomization with the computer-generated random numbers) divided into four groups and were sedated with the corresponding drug intravenously. Horses belonged to the group R received 0.04 mg/kg of romifidine (Sedivet[3]). Patients of the group D were sedated with 15 µg/kg of detomidine (Domosedan[4]). Animals of the group DB received 10 µg/kg of detomidine combined with 10 µg/kg of butorphanol (Nargesic[5]). Horses of the group X were sedated with 0.7 mg/kg of xylazine (Megaxilor[6]).

Data analysis

The data were analyzed with ANOVA by means of the general linear model (GLM) procedure in SAS Version 9.4[7]. The fixed factors included sedation protocol (four levels:

romifidine, detomidine, detomidine combined with butorphanol, and xylazine), time (seven levels: before sedation, 5, 15, 30, 60, 120 and 180 minutes after the administration of sedation), sex (three levels: intact females, intact males, spayed males) and eye (two levels: right eye, left eye). The age (years) and weight (kg) of the horses were considered covariates. The STT I values were reported as least-squares means (LSMeans) ± standard error of the mean (SEM). The age and weight were expressed as mean ± standard deviation (SD). *P* values < 0.05 were considered significant.

RESULTS

Forty adult Italian saddle horses were included in the study. Each group consisted of 10 subjects (Table 1). The STT I values are reported in table 2.

In the group R, there were no significant differences in the STT I values.

In the group D, the STT I value was significantly lower 15 minutes after the administration of sedation compared to the values before sedation ($P = 0.007$), at 5 minutes ($P = 0.001$) and 180 minutes ($P = 0.001$).

In the group DB, the STT I value at 30 minutes was significantly lower compared to the values before sedation ($P = 0.004$), at 120 minutes ($P = 0.046$) and 180 minutes ($P = 0.046$). The STT I value at 60 minutes was significantly lower compared to the values before sedation ($P = 0.001$), at 5 minutes ($P = 0.006$), 15 minutes ($P = 0.024$), 120 minutes ($P = .001$) and 180 minutes ($P = 0.001$).

In the group X, the STT I values at 5, 15 and 30 minutes were significantly higher compared to the value before sedation ($P = 0.001$). The STT I values at 5 and 15 minutes were significantly higher compared to those at 60 minutes ($P_5 = 0.029$, $P_{15} = 0.001$), 120 minutes ($P_5 = 0.013$, $P_{15} = 0.001$) and 180 minutes ($P_5 = 0.021$, $P_{15} = 0.001$). The STT I value at 30

minutes was significantly higher compared to those at 5 minutes ($P = 0.038$) and 120 minutes ($P = 0.001$).

With regard to sex and right eye and left eye, no significant differences were recorded.

DISCUSSION

This study demonstrates that the administration of detomidine alone or combined with butorphanol decreases tear production. Intravenous romifidine does not affect tear production, whereas the administration of xylazine is associated with increased aqueous tear production.

The choice of the most suitable α2-adrenoceptor agonist for sedation is critical for the success of the ocular examination (Hendrix 2005). Detomidine is often used because it ensures a steady head position even though it is associated with decreased tear production (Ghaffari *et al.* 2017). As expected, decreased tear production was recorded 15 minutes after sedation because the peak of the sedative effects of detomidine is reached within 15 minutes after intravenous administration (Muir 2009). Nevertheless, differently from previous findings (Ghaffari *et al.* 2017), the significant decrease in tear production is no longer present 30 minutes after sedation. Based on the dose-related effects of detomidine (Muir 2009), the probable reason is that the dose employed in the present study is lower compared to that used by Ghaffari et *al.* (2017).

One explanation for the decreased measurable tear production after the administration of detomidine is the evaporative losses increased by inadequate blinking caused by sedation (Crispin 2000).

Nevertheless, the most likely causes for the reduction in aqueous tear production are the neurophysiological mechanism and hemodynamic changes. Even though specific details of the innervation of equine lacrimal glands are poorly known (Crispin 2000), it is likely that the

postsynaptic activation of α2-adrenoceptors in the central nervous system may have decreased basal tear production. Furthermore, the reduction in the STT I values may be due to the decrease in reflex tear production mediated by diminished nociceptive transmission, which is modulated by α2- adrenoceptors (Martin 2005).

With regard to hemodynamic changes, detomidine induces mild hypotension that is responsible for decreased perfusion of the lacrimal glands followed by a consequent decrease in the STT I values (Muir 2009). Detomidine could have additionally caused a direct vasoconstriction of the vessels of lacrimal glands.

Intravenous administration of the combination of detomidine and butorphanol is associated with a more accentuated and prolonged decrease in tear production compared to that induced by detomidine alone. Furthermore, horses belonged to the group DB showed the lowest STT I values recorded in the present study even though the STT I values do not go below the reference range reported in normal horses (Marts *et al.* 1997; Beech *et al.* 2003; Piccione *et al.* 2008). These observations agree with those of similar studies in dogs (Dodam *et al.* 1998; Sanchez *et al.* 2006) and underscore that opioidergic signalling pathways plays an important role in tear production (Zagon *et al.* 2012). Moreover, it is likely that detomidine combined with butorphanol may have additive and /or synergistic effects in decreasing equine tear production improving analgesia and affecting hemodynamic changes and neurophysiological mechanism (Muir 2009; Bianchi *et al.* 2015).

Another possible reason for the decreased measurable tear production caused by the combination of detomidine and butorphanol is a potential opioid-induced alteration of the metabolism of lacrimal glands (Mouney *et al.* 2011). Nevertheless, further studies are needed to clarify the effect of intravenous butorphanol alone on tear production.

An ocular examination is usually a short procedure and romifidine is not therefore the most suitable α2-adrenoceptor agonist because its duration of action is longer compared to those of

185 detomidine and xylazine (Muir 2009). Furthermore, based on a previous study demonstrating
186 that 0.04 mg/kg of romifidine reduced intraocular pressure in horses (Marzok *et al.* 2014), we
187 expected that the administration of romifidine was associated with decreased tear production.
188 Contradicting this expectation, romifidine did not affect the STT I measurements. The
189 probable reason for these results is that the dose of romifidine employed in the present study
190 is too low to affect aqueous tear production. Even though α2-adrenoceptor agonists
191 commonly induce hemodynamic changes in a dose-dependent manner (Nannarone *et al.*
192 2007), we supposed that 0.04 mg/kg of romifidine did not markedly reduce cardiac output in
193 horses enrolled in this study, as it could have certainly happened with higher doses (Peboni
194 Figueiredo *et al.* 2005). Consequently, it is likely that a regular blood flow to lacrimal glands
195 was provided with no changes in the STT I values.
196 The effect of intravenous xylazine on tear production in horses has not been clearly
197 identified. It seems that xylazine alone has no direct effects on tear production (Marts *et al.*
198 1977), whereas intravenous administration of 150 mg of xylazine is associated with
199 decreased tear production in anesthetized horses ranged in age from 6 months to 14 years
200 (Brightman *et al.* 1983). Unfortunately, Brightman *et al.* (1983) did not report the dose of
201 xylazine expressed as mg/kg. However, our results contradict previous findings because
202 intravenous xylazine unexpectedly increased the STT I values. There are no reports in the
203 veterinary literature that can explain our results. It is likely that the increased measurable tear
204 production was due to ataxia, excitation, movement and the head “jerks” caused by xylazine
205 (Gilger *et al.* 2011). We suggest that this behavior may have increased complete blink rate
206 and, consequently, tear production (Crispin 2000). However, further studies are needed to
207 confirm this hypothesis.
208 With regard to the influence of sex and right or left eye on tear production, our results agree
209 with previous findings (Crispin 2000; Beech *et al.* 2003). In fact, in the present study, sex did

not influence the STT I values (Beech *et al.* 2003) and the second eye was not affected by the stimulation of basal secretion in the first eye (Crispin 2000).

The present study shows some limitations related to environmental conditions, equipotency of α2-adrenoceptor agonists and head position.

The influence of time of day, season, and environment on tear production has not been entirely clarified by researchers (Beech *et al.* 2003; Piccione *et al.* 2008). These factors do not seem to influence the STT I values even though a circadian rhythm of tear production was reported (Beech *et al.* 2003; Piccione *et al.* 2008). In the present study, the influence of these variables on the STT I values was not investigated.

Another limitation of the study is that we did not accurately evaluate the equipotency of α2-adrenoceptor agonists. We chose approximate equipotent doses based on the veterinary literature (England *et al.* 1992; Nannarone *et al.* 2007).

Head position and height of the eye above the level of the heart affect intraocular pressure but no report investigated the influence of these variables on tear production (Holve 2012). Consequently, we cannot clearly exclude an influence of these factors on the STT I measurements.

In conclusion, intravenous administration of romifidine does not affect aqueous tear production and may be useful to measure tear production in horses undergoing ophthalmic procedures requiring sedation. Xylazine increases tear production and is not suitable for chemical restraint to perform a complete ocular examination in horses. The administration of detomidine alone or combined with butorphanol reduce the STT I values and the use of ophthalmic ointments is therefore recommended after sedation.

AUTHORS' DECLARATION OF INTERESTS

No conflicts of interest have been declared.

ETHICAL ANIMAL RESEARCH

This study was performed in accordance with the Legislative Decree n. 26 of 4th March 2014 under Italian Animal Welfare Legislation and was approved by the Institutional Ethics Committee for animal welfare of the University of Parma.

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AUTHORSHIP

F. Leonardi, B. Simonazzi, M. Dubau and M. Angelone designed the study. G.L. Costa and A. Sabbioni undertook the statistical analyses. All authors contributed and commented to the manuscript and read and approved the final version.

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TABLES

Table 1. Sex, age and body weight of horses belonged to the four groups

	Group R (n = 10)	Group D (n = 10)	Group DB (n = 10)	Group X (n = 10)
Sex				
Number of intact females	6	3	2	6
Number of intact males	2	3	3	2
Number of spayed males	2	4	5	2
Age (year)				
Mean ± standard deviation	23.3 ± 6.46	12.3 ± 7.64	15.8 ± 7.99	22.5 ± 6.45
Range	15-33	3-22	3-33	15-33
Body weight (kg)				
Mean ± standard deviation	492.5 ± 46.70	429.8 ± 54.32	500.8 ± 64.62	488 ± 36.80
Range	426-574	351-506	394-608	430-570

R = romifidine; D = detomidine; DB = detomidine combined with butorphanol; X = xylazine; n = number of horses

Table 2. STT I values (mm/min) before sedation and at 5, 15, 30, 60, 120 and 180 minutes after the administration of sedation in the four groups. STT I values are expressed as least-squares means ± standard error of the mean (SEM)

	Before sedation	5 min	15 min	30 min	60 min	120 min	180 min	SEM
Group R	24.18	24.23	25.18	24.83	23.33	22.88	23.93	1.00
Group D	21.48^{b}	20.79^{a}	$18.17^{a,b,c}$	20.17	19.33	20.44	22.51^{c}	0.975
Group DB	$21.06^{a,c}$	19.27^{b}	18.69^{d}	$17.44^{c,f,n}$	$15.81^{a,b,d,e,r}$	$19.99^{n,r}$	$20.44^{e,f}$	0.988
Group X	$20.88^{a,b,c}$	$25.17^{a,d,f,h,i}$	$26.72^{b,e,l,m}$	$28.07^{c,f,g}$	$22.13^{d,e}$	$21.73^{g,h,l}$	$21.93^{i,m}$	0.999

R = romifidine; D = detomidine; DB = detomidine combined with butorphanol; X = xylazine; min = minutes

In the same row, the same superscript (^{a,b,c,d,e,f,g,h,i,l,m,n,r}) marks the values with a statistically significant difference between them ($P < 0.05$).