COMPARATIVE EVALUATION OF THE INTRAVENOUS EFFECT OF MEDETOMIDINE, TRAMADOL AND MEDETOMIDINE/TRAMADOL COMBINATION ON TEAR PRODUCTION IN CLINICALLY HEALTHY DONKEYS (EQUUS ASINUS)

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Summary


Various ophthalmic disorders (conjunctivitis, corneal wounds, keratitis) have been reported in donkeys. There are no studies on the effect of medetomidine or tramadol on Schirmer tear test (STT) readings in donkeys. This prospective study investigated changes in STT readings in 24 clinically healthy donkeys (Equus asinus) (14 geldings and 10 mares) treated with commonly used doses of medetomidine hydrochloride and tramadol hydrochloride as mono- or combined therapy. Analgesia, sedation, ataxia, and STT readings were measured before treatment (baseline) and at different periods after administration (5–120 min) of the specific drug in each group. Tramadol monotherapy induced a mild analgesic effect (score 1) at 10 min post-administration. All treated donkeys exhibited mild to moderate ataxia. Medetomidine alone or in combination with tramadol induced a significant decrease (P<0.05) in the STT readings in both right and left eyes at 5, 15, 30, and 60 min relative to baseline, and the lowest values were observed 60 min after drug administration in both groups. Intravenous administration of medetomidine alone or in combination with tramadol induced a significant reduction in STT readings in clinically healthy donkeys. Therefore, in donkeys, the ocular surface treated with these sedatives should be carefully examined and adequately covered by an artificial tear solution or ophthalmic gel.

Key words: combination therapy, donkey, medetomidine, tear production, tramadol
INTRODUCTION

Donkeys (*Equus asinus*) are used on a wide scale by farmers in Egypt. Humans have relied on donkeys as workhorses for various activities, such as cultivating land and transporting humans and goods (Monti *et al*., 2012). Owing to their small size, capacity to survive on poor-quality diets, and reduced demand for feed and drink, donkeys are the best draught animals (Gilger & Stoppini, 2005). Globally, in recent years, focus on the welfare of donkeys has been growing as donkey’s milk is being given to children with intolerance to cow’s milk (Monti *et al*., 2012).

Generally, a good performance by animals requires them to have normal vision. In donkeys, various ophthalmic disorders, including conjunctivitis, corneal wounds, and keratitis, have been reported (Leonardi *et al*., 2018).

Tears are critical for the eyes as they extract and remove debris and bacteria mechanically and lubricate the conjunctiva (Misk, 1990). The Schirmer tear test (STT) is one of the most effective eye tests used for accurate diagnosis of dry eyes in horses (Gilger & Stoppini, 2005) and is considered the most widely used method for evaluating basal and reflex tear production in horses without eye anaesthetic administration (Borhani *et al*., 2021). STT readings lower than 10 mm/min are considered pathological. However, values higher than 35 mm/min are not pathological (Hendrix, 2005). The STT should be performed before eye manipulation during an eye examination to avoid a tearing reflex (Gilger & Stoppini, 2005). The higher corneal sensitivity in horses is vital to assess the equine eye but with suitable restraint (Alizadeh *et al*., 2021). Thus, sedation may be necessary, and the effectiveness of sedation on STT readings should be considered (Hendrix, 2005).

Generally, standing sedation greatly helps in proper eye checkup and several eye procedures in horses and increases protection for both the equine patient and the inspector. In horses, medetomidine is an alpha-2-adrenoceptor agonist commonly used as a sedative for a thorough eye examination (Holve, 2012). Medetomidine hydrochloride tranquilisation is often preferred for an eye checkup in horses as it offers a powerful sedative and analgesic effect compared to xylazine and detomidine (Verbruggen *et al*., 2000; Creighton *et al*., 2012). Because of its long half-life, medetomidine is widely used as a premedication before general anaesthesia (Kanda *et al*., 2015). For a painful eye, combining an alpha-2-adrenoceptor agonist with an opioid to obtain a superior analgesic effect is recommended (Hendrix, 2005; Muir, 2009).

In dogs, medetomidine alone or in combination with butorphanol results in a substantial reduction in tear production 15 min after sedation (Di Pietro *et al*., 2021). Intramuscular medetomidine induces a significant decline in STT readings in both eyes in pigs (Kanda *et al*., 2019a), cats (Kanda *et al*., 2019b), and rats (Kanda *et al*., 2020). Many alpha-2-adrenoceptor agonists used alone or in combination with other medications substantially decrease tear flow in horses (Ghaffari *et al*., 2017; Leonardi *et al*., 2018), dogs (Dodam *et al*., 1998; Leonardi *et al*., 2019), and cats (Di Pietro *et al*., 2016).

Tramadol is a synthetic analgesic medication that is a codeine equivalent. Because of its analgesic effect, it was recently used to treat extreme post-operative pain in veterinary medicine (Pypendop *et al*., 2009). Tramadol has minor effects on
gastrointestinal motility and cardiorespiratory function and the same analgesic effects as morphine (Natalini & Robinson, 2000). Tramadol has no analgesic effect on horses at any of the doses assessed (Dhanjal et al., 2009; Franco et al., 2014). A combination of xylazine or detomidine with tramadol has sedative or analgesic effects and can be used in standing horses for diagnostic and simple operations, with careful monitoring for shortlly excited behaviour (Seo et al., 2011; Kim et al., 2012). Tear production in dogs is not affected by tramadol (Santos et al., 2013; Ruiz et al., 2015). Some studies have identified the effect of medetomidine on STT readings in pigs (Kanda et al., 2019a), cats (Kanda et al., 2019b), rats (Kanda et al., 2020), and dogs, either alone or in combination with opioids (Sanchez et al., 2006), but there is no documentation of the effect of medetomidine or tramadol on STT readings in donkeys. We hypothesised that our results would be in close agreement with previous studies; medetomidine alone or in combination with tramadol substantially decrease tear flow in donkey. Thus, this prospective study examined changes in

STT readings associated with clinically current doses of medetomidine, tramadol, and the medetomidine/tramadol combination in clinically healthy donkeys and reported these changes within 120 min of administration.

MATERIALS AND METHODS

Ethical statement

Before the trial, a full physical examination (heart rate, respiratory rate and rectal temperature) and regular haematological (CBC) and biochemical tests (ASAT, ALAT, total protein, creatinine) were performed (Table 1) to prove the normal status of the donkeys. Donkeys included in the current study had no history of ocular abnormalities upon ophthalmic examination including indirect ophthalmoscopy and fluorescein staining test. Donkeys with abnormalities of the ocular surface or with STT I values lower than 15 mm/min were excluded from our study. This study was approved by the Animal Care Committee of Mansoura University, Egypt, in compliance with Egyptian ethical codes for studies on animals (approval no. 04-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal saline (n=6)</th>
<th>Tramadol (n=6)</th>
<th>Medetomidine (n=6)</th>
<th>Combination (n=6)</th>
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<tbody>
<tr>
<td>Heart rate (min⁻¹)</td>
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<td>45–48</td>
<td>47–49</td>
<td>47–50</td>
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<td>16–23</td>
<td>20–22</td>
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<td>37.5–37.6</td>
<td>37.7–37.9</td>
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<tr>
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<td>5.7–7.22</td>
<td>4.5–6.55</td>
<td>4.51–7.1</td>
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<tr>
<td>Haemoglobin (g/L)</td>
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<td>102–146</td>
<td>98–136</td>
<td>112–141</td>
</tr>
<tr>
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<td>9–23</td>
<td>11–27</td>
<td>11–22</td>
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<td>290–324</td>
<td>259–411</td>
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<tr>
<td>Total protein (g/L)</td>
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<td>64–68</td>
<td>59–71</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
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<td>88.4–105</td>
<td>70.7–92.7</td>
<td>79.5–114</td>
</tr>
</tbody>
</table>
Comparative evaluation of the intravenous effect of medetomidine, tramadol and medetomidine ...
and marked stumbling and walking); and 3 = deep sedation (clear lethargy, head droop, and failure to respond to external stimuli; recumbence or falling during walking). The degree of ataxia was ranked from 0 to 3 as per Hamed et al. 2017: 0 = normal; 1 = unimportant (minor stumbling but easily able to walk again); 2 = moderate (noticeable stumble and clear ataxic walk); and 3 = extreme (recumbency or falling while walking). In all cases, the same investigator measured antinociception, sedation, and ataxia but was ignorant of the medication used. The STT readings, degree of antinociception, and sedation were measured before sedation baseline (T0) and then at 5 (T5), 15 (T15), 30 (T30), 60 (T60), 90 (T90), and 120 (T120) min post-sedation. The experimental donkeys were clinically monitored for 1 week post-treatment.

Schirmer tear test

STTs were carried out under the same environmental conditions in indoor-inaudible traditional housing locations. Measurements were taken by the same investigator at a set time of the day (8:00–11:00 a.m.) to eliminate human and diurnal variations, and he was blinded from the treatment being performed (Piccione et al., 2018). An individual, sterile, graduated 5 mm × 35 mm STT strip (I-DEW Tear Strips, Schirmer Strips, 100 Sterile Strips; Entod Research Cell UK Ltd., London, UK) was inserted into the lower eyelid around one-third of the distance between the temporal canthus and the nasal canthus for 1 min without any topical anaesthetic. The investigator’s hands were clean and dry when taking the strip out of its box. The length of the moistened area on the strip was recorded in mm/min. For every donkey, the STT was performed randomly (right eye versus left eye).

Statistical analysis

Data processing was conducted using SPSS Statistics (SPSS for Windows, version 16.0; SPSS Inc., Chicago, IL, USA). Data for normal distribution were initially analysed using the Kolmogorov–Smirnov method. Typically, data were distributed; thus, the mean and standard deviation (SD) for each variable were determined at each point in time. Analysis of variance (ANOVA) was conducted to assess the key effects of time and treatment using a general linear model of repeated steps. Wilks’s lambda test was performed to determine interactions within the group and evidence of seven time interactions in the treatment; when the test showed a statistically significant difference between groups, a t-test was performed to determine which group was statistically different at each point in time. The difference between the means at P<0.05 was considered statistically significant.

RESULTS

In this study, after intravenous (IV) administration of medetomidine and tramadol, whether mono- or combination therapy, the clinical parameters and mean STT readings in the right and left eyes were determined in clinically healthy donkeys. Following-up the cases did not prove any signs of ocular abnormalities upon ophthalmic examination including indirect opthalmoscopy and fluorescein staining test. No harmful or neurological symptoms were noted in the donkeys 1 week post-administration.

Antinociception onset was noted in groups M and MT within 5 min post-administration compared with groups T and C. Complete analgesia was induced from T5 to T30 (score 3) and mild to
Comparative evaluation of the intravenous effect of medetomidine, tramadol and medetomidine ...

moderate analgesia – from T60 to T90 (Fig. 1). Groups M and MT had a significantly higher (P=0.017) analgesic score from T5 to T120 compared with groups T and C. Group T showed a mild analgesic effect, based on the pinprick test (score 1), at ~10 min post-administration. However, during the whole study, group C did not exhibit analgesia (Fig. 1).

Once the donkeys received normal saline, none of the sedation signs (score 0) were observed. The sedative effect was evident in groups M and MT within 5 min compared to groups T and C. The sedation score was approximately deep (score 3) in the first 15 min, moderate (score 2) from T30 to T60, mild at T90, and returned to T0 at T120. Groups M and MT showed a significantly higher (P= 0.004) sedation score from T5 to T90 compared to groups T and C (Fig. 2). Of note, for ~10 min after tramadol administration, a mild sedative effect was observed (score 1) (Fig. 2). Donkeys in groups M, MT, and T showed mild-to-moderate ataxia, which was observed from T5 to T30 and persisted in groups M and MT for T60. Conversely, group C did not show any signs of ataxia during the study (Fig. 3).

The STT (mm/min) mean ± SD baseline readings for all groups for both eyes were approximately identical and within the reference range. STT readings (mean ± SD) for both eyes of groups M and MT at T5, T15, T30, T60, and T90 showed a substantial decrease compared to baseline (P<0.0001, Wilks’s lambda test for drug × time interaction). Notably, the largest decrease in STT readings in groups M and MT was detected in both eyes at T60 (12.10 ± 0.7 and 11.0 ± 0.8 mm/min for group M and 12.0 ± 0.6 and 11.10 ± 0.7 mm/min for group MT for the right and the left eye, respectively) (Table 2). At T20, the decrease in the STT reading for both eyes began to slowly increase toward
Fig. 2. Sedation values (mean±SD) after intravenous administration of medetomidine (0.007 mg.kg⁻¹), tramadol (2 mg.kg⁻¹), medetomidine/tramadol combination, and normal saline 0.9% in clinically healthy donkeys. Variables with different superscripts in the same column are significantly different at P<0.05.

Fig. 3. Ataxia values (mean±SD) after intravenous administration of medetomidine (0.007 mg.kg⁻¹), tramadol (2 mg.kg⁻¹), medetomidine/tramadol combination, and normal saline 0.9% in clinically healthy donkeys. Variables with different superscripts in the same column are significantly different at P<0.05.
Comparative evaluation of the intravenous effect of medetomidine, tramadol and medetomidine ... baseline (Table 2). In group T, there was no substantial differences (P<0.05) in the STT readings for both eyes compared with baseline and group C (Table 2). In group S, there was no substantial difference (P>0.05) between baseline and post-treatment STT readings (Table 2). Unfortunately, some donkeys exhibited side effects post-administration in groups M (one salivation and two penile prolapses), MT (one salivation, two penile prolapses, and one excitation), and T (two excitations and two muscle tremors).

DISCUSSION

Appropriate chemical restraint is vital to perform an STT and a proper eye check-up. It increases protection, particularly in the case of a painful eye, for both the equine patient and the investigator (Hendrix, 2005). Choosing the most appropriate alpha-2-adrenoceptor agonist for sedation is crucial to the treatment of problems associated with equine dry eye, as such medications can affect STT readings (Hendrix, 2005). Medetomidine is often recommended for an eye checkup in horses as it has a strong sedative and analgesic effect compared with xylazine and detomidine (Creighton et al., 2012). To obtain a superior analgesic effect for a painful eye, it may be possible to combine an alpha-2-adrenoceptor agonist with opioids (Hendrix, 2005; Muir, 2009), and the combination of medetomidine (α-2

Table 2. Schirmer tear test results in the left and right eyes after intravenous administration of medetomidine (0.007 mg kg⁻¹), tramadol (2 mg kg⁻¹), medetomidine/tramadol combination, and normal saline 0.9% in clinically healthy donkeys. Data are presented as mean ± SD (n=6)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time post-treatment (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Left eye</td>
<td></td>
</tr>
<tr>
<td>Normal saline</td>
<td>19.0±</td>
</tr>
<tr>
<td></td>
<td>1.7ᵃ</td>
</tr>
<tr>
<td>Tramadol</td>
<td>18.6±</td>
</tr>
<tr>
<td></td>
<td>0.8ᵃ</td>
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<tr>
<td>Medetomidine</td>
<td>20.3±</td>
</tr>
<tr>
<td></td>
<td>1.2ᵃ</td>
</tr>
<tr>
<td>Combination</td>
<td>18.1±</td>
</tr>
<tr>
<td></td>
<td>0.7ᵇ</td>
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<tr>
<td>Right eye</td>
<td></td>
</tr>
<tr>
<td>Normal saline</td>
<td>19.5±</td>
</tr>
<tr>
<td></td>
<td>1.3ᵇ</td>
</tr>
<tr>
<td>Tramadol</td>
<td>19.16±</td>
</tr>
<tr>
<td></td>
<td>1.1ᵇ</td>
</tr>
<tr>
<td>Medetomidine</td>
<td>21.5±</td>
</tr>
<tr>
<td></td>
<td>0.8ᵃ</td>
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<tr>
<td>Combination</td>
<td>17.5±</td>
</tr>
<tr>
<td></td>
<td>0.5ᵇ</td>
</tr>
</tbody>
</table>

Variables with different superscript letters in the same column are significantly different at P<0.05. MANOVA fit, P<0.0001. Wilks’ Lambda test for drug × time interaction, P<0.0001.
agonist) and tramadol (opioid) may enable satisfactory ocular surgery in a standing position in donkeys.

In normal horses, the mean STT readings range from 12.7 to 24.8 mm wetting/min (Williams et al., 1979). In this study, the baseline STT readings for all treated donkeys were within the reference range (17.5–21.5 mm/min). IV administration of medetomidine resulted in a statistically significant decrease (P<0.05) in STT readings in donkeys. This decrease was evident from 5 min and was maintained for up to 90 min post-administration. The peak reduction in STT readings was observed 5 min after the IV administration of detomidine. Our findings were in agreement with those of similar studies following medetomidine administration in dogs and cats (Ghaffari et al., 2010; Sanchez et al., 2006).

The exact technique by which medetomidine can reduce tear production is still unclear. There are three possible explanations: (i) increased evaporation due to inadequate blinking resulting from the effect of sedation (Crispin, 2000); (ii) postsynaptic motivation of alpha-2-adrenoceptors in the central nervous system (CNS), which may play a vital role in lowering the production of basal tears (Dodam et al., 1998; Leonardi et al., 2018) and a decrease in the output of reflex tears mediated by reduced nociceptive transmission, organised by the alpha-2-adrenoceptor; and (iii) alpha-2-agonist-induced adequate hypotension, leading to reduced tear gland perfusion, followed by a consequent fall in STT readings (Muir, 2009; Leonardi et al., 2018).

In this study, donkeys treated with medetomidine/tramadol combination therapy showed a significant (P<0.05) decrease in tear production. This finding is consistent with that recorded in dogs (Dodam et al., 1998; Leonardi et al., 2019; Sanchez et al., 2006) and horses (Leonardi et al., 2018). The evaporative loss caused by the sedative effects of medetomidine/tramadol combination is an obvious cause of decreased measurable tear production, which decreases blinking. Consequently, the tear film’s aqueous layer can evaporate more (Sanchez et al., 2006). A previous study (Leonardi et al., 2018) added that reducing STT readings is a possible alteration in the metabolism of lacrimal glands caused by a combination of detomidine and butorphanol triggered by opioids.

IV administration of tramadol (2 mg/kg) did not affect the STT readings in donkeys. This may be due to a lack of the antinociceptive effect of tramadol in donkeys, as antinociception has also been identified as a possible cause of decreased tear production (Dhanjal et al., 2009; Dodam et al., 1998). Similar results were observed following doses of 2 mg/kg (Santos et al., 2013) and 4–6 mg/kg tramadol in dogs (Ruiz et al., 2015). However, Santos et al. (2013) reported that the tramadol/acepromazine combination significantly reduces tear production in dogs. On the contrary, tear production declined significantly in dogs after intramuscular administration of 1 mg/kg of morphine compared to baseline (Mouney et al., 2011).

Donkeys treated with medetomidine and medetomidine/tramadol combination showed satisfactory analgesia. Medetomidine is a potent and complete alpha-2-adrenoceptor agonist, and its selectivity to a-2/a-1 is greater than that of xylazine (Creighton et al., 2012). The antinociceptive effect of medetomidine is triggered by a diminishing release of epinephrine and norepinephrine, which play a vital role in pain sensation by inhibiting the sympathetic nervous system through their action.
on α-2 receptors (Ambrisko & Hikasa, 2002). In group T, tramadol induced a minor analgesic effect for up to 10 min post-administration, as described by Seo et al. (2011) and Kim et al. (2012). There was no noticeable difference in the duration of analgesia in groups M and MT. Conversely, in horses, the addition of tramadol to xylazine or detomidine has a longer effect compared to any sole drug (Seo et al., 2011; Kim et al., 2012). Studies have shown that there is no analgesic effect of tramadol in horses (Dhanjal et al., 2009; Franco et al., 2014).

In this study, groups M and MT showed moderate-to-deep sedation of equal duration. The sedative effect of medetomidine is due to a reduction in sympathetic CNS outflow (Toutain et al., 1982). These findings correlate with those of Kim et al. (2012), who noted that the analgesic effect of the detomidine/tramadol combination in horses is similar to the effect of detomidine alone. Another study reported that the xylazine/tramadol combination has a longer sedative effect on horses than xylazine alone (Seo et al., 2011). However, tramadol has a mild sedative effect for a short period on horses (Seo et al., 2011; Kim et al., 2012). Furthermore, injections of tramadol at a dose of 2 mg/kg do not induce sedation in horses (Dhanjal et al., 2009). Despite the reduced sedative and analgesic effects of IV administration of tramadol, its epidural injection provides prolonged analgesia without CNS agitation and motor activity and behavioural changes (Natalini & Robinson, 2000).

Groups M, MT, and T showed mild to moderate ataxia. This could be attributed to the effect of medetomidine (Ambrisko & Hikasa, 2002) and tramadol (Dhanjal et al., 2009; Franco et al., 2014). On the contrary, no ataxia was recorded after IV administration of tramadol in horses (Kim et al., 2012). In horses, IV administration of tramadol does not produce the locomotor motivation noted with other opioids, but other CNS excitations, such as more excited temper and behaviour, increased sensitivity to noise and stimulation, tremor, and head nodding, are observed (Dhanjal et al., 2009). However, in this study, tramadol had no undesirable effects on donkeys. Giorgi et al. (2007) reported that increasing the tramadol dose up to 5 mg/kg IV prompts tremor, confusion, excitement, and tachycardia.

This study had several limitations. First, future studies are required to evaluate the effects of kinetic analyses using various doses of medetomidine and tramadol on tear production in donkeys. We selected approximately current doses based on the previous veterinary literature on horses and donkeys (Dhanjal et al., 2009; Arican et al., 2015; Hamed et al., 2018). Second, the sample size was small, which may not allow a definitive conclusion. Subsequently, more research using large samples of donkeys is needed. Third, we did not examine the effect of medetomidine and tramadol, whether mono- or combination therapy, on the STT readings of donkeys with ophthalmic diseases. Therefore, further research is required to investigate this shortcoming.

CONCLUSIONS

IV administration of medetomidine either alone or in combination with tramadol can be used for diagnostic techniques and minor ophthalmic surgeries in standing donkeys. Such combination therapy shows a significant decline in STT readings in clinically healthy donkeys. Accordingly, the ocular surface of donkeys treated with these sedatives should be carefully in-
spected and satisfactorily covered by an artificial tear solution or opthalmic gel.

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Comparative evaluation of the intravenous effect of medetomidine, tramadol and medetomidine ...


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