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ORIGINAL RESEARCH

A comparison of immobilisation quality and cardiorespiratory effects of etorphine-azaperone versus etorphine-midazolam combinations in blesbok

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The study compared immobilisation of blesbok (*Damaliscus pygargus phillipsi*) with etorphine and azaperone vs etorphine and midazolam. Twelve female blesbok, weighing 59.4 ± 2.8 kg, were used. Each animal randomly received Treatment 1 (T1) (etorphine, 0.07 ± 0.003 mg/kg + azaperone, 0.36 ± 0.02 mg/kg) and Treatment 2 (T2) (etorphine, 0.07 ± 0.003 mg/kg + midazolam, 0.20 ± 0.01 mg/kg) with a one-week washout period between treatments. Induction times were recorded followed by physiological monitoring for 45 minutes of immobilisation. Immobilisation was reversed with naltrexone (20 mg per mg etorphine). Recovery times were also recorded. Induction, immobilisation and recovery were scored with subjective measures. Inductions and recoveries did not differ between combinations, but the quality of immobilisation was significantly better with T1. Rectal temperature and blood pressure were significantly lower during T1. Both treatments resulted in severe hypoxaemia and impaired gas exchange, although overall hypoxaemia was more pronounced for T1. Animals treated with T2, however, exhibited a deterioration in respiration as the monitoring period progressed, possibly as a result of impaired ventilatory muscle function due to the effects of midazolam. Both combinations are suitable for adequate immobilisation of blesbok and should be selected based on the specific capture situation. Supplementation with oxygen is highly recommended.

Keywords: azaperone, midazolam, blesbok, cardiorespiratory function, chemical immobilisation, etorphine

Introduction

Southern African antelope species are most commonly immobilised using drug combinations consisting of anaesthetics, sedatives and tranquilisers since these result in reduced stress and injuries compared to manual restraint (Hampton et al. 2020). In a free-range setting these combinations are most often opioid-based and may include the addition of a butyrophenone tranquiliser or alpha-adrenergic sedatives (Kock & Burroughs 2012). More recently, the use of benzodiazepine sedatives has become increasingly popular in wildlife species, although little research has been done on their effects when used in chemical immobilisation combinations in ungulates (Adami & Wenker 2013; Curro et al. 2004; Kalema-Zikusoka et al. 2003; King et al. 2008; Lafortune et al. 2005; Lapid & Shilo-Benjamini 2015; Stegmann & Jago 2006; Van Zijll Langhout et al. 2016; Wolfe & Miller 2016).

Etorphine is a potent opioid that is widely used for the immobilisation of southern African ungulate species (Alford et al. 1974; Fahlman 2008; Kock & Burroughs 2012). It has a rapid onset and relatively short duration of action (Blane et al. 1967; DeRossett & Holtzman 1984). Etorphine's use in immobilising drug combinations is popular because of its ability to induce reversible catatonic immobilisation and analgesia at relatively low doses compared to other anaesthetics, and because its high potency allows for the administration of small volumes via projectile darts (Blane et al. 1967; Riviere & Papich 2009; Woodward 2009).

Azaperone is a member of the butyrophenone family of tranquilisers (Clarke et al. 2014; West et al. 2007). The butyrophenones are neuroleptic drugs that have their main effects mediated via dopaminergic (D_2) antagonism, resulting in tranquilisation and the potentiation of immobilisation and anaesthesia (Gross 2001; Kock & Burroughs 2012; Riviere & Papich 2009; Swan 1993). The butyrophenones also have antagonistic activity against α_1-adrenergic, histaminergic and cholinergic receptors although the latter two interactions are reported to induce mild effects (Riviere & Papich 2009). Azaperone has a relatively fast onset but a short duration of action compared to long-acting neuroleptic tranquilisers, and is frequently used in combination with potent opioids in the immobilisation of many wildlife species, or on its own as a short-acting neuroleptic tranquiliser in wild herbivores (Clarke et al. 2014; Marco et al. 2010; Portas 2004; Read 2002; West et al. 2007). It causes peripheral vasodilation by blocking the α_1-adrenergic receptors, which results in striated muscle relaxation in arterioles (Mentaberre et al. 2010). This effect is often beneficial in wildlife immobilisation as it reduces the hypertensive effects of opioids and alpha$_2$-agonists (Bothma 1990; Knox 1992; Marco et al. 2010; Meyer et al. 2008).

Midazolam is a short-acting benzodiazepine sedative that is becoming increasingly popular in veterinary medicine. It is

currently only registered for human use, both in South Africa and worldwide. Its popularity is largely due to its anxiolytic, psycho-sedative, hypnotic, anticonvulsant, muscle relaxant and anterograde amnestic effects (Henry et al. 1998; Papich 2016). Its solubility and unique pH-dependent molecular structure account for many of midazolam's desirable characteristics (Nordt & Clark 1997). In parenteral preparations, midazolam has a pH of 3.5 and is a water-soluble, relatively non-irritating solution that is rapidly absorbed from the injection site (Clarke et al. 2014; Henry et al. 1998; Reves et al. 1985; Schwartz et al. 2013). At physiological pH, midazolam is highly lipophilic, which facilitates its movement across the blood–brain barrier into the central nervous system (CNS), which accounts for its rapid onset of action (Henry et al. 1998; Reves et al. 1985). The effects of midazolam on the CNS are dependent on the dose administered, the route of administration, and the presence or absence of other medications (Fresenius Kabi 2017). Within the CNS, it exerts its activity at the benzodiazepine receptor (Nordt & Clark 1997). The benzodiazepine receptor is part of a supramolecular complex containing the amino acid neurotransmitter gamma-aminobutyric acid $(GABA)_A$ receptor and the chloride channel. In this complex, the benzodiazepines act allosterically, i.e. they modify the GABA-binding sites and increase the action of GABA on nerve cells (Amrein et al. 1988; Nordt & Clark 1997; Papich 2016). The sedative effects of midazolam may be attributed to the potentiation of GABA pathways that act to regulate release of monoamine neurotransmitters in the CNS. Benzodiazepines like midazolam may also act as muscle relaxants by inhibiting certain spinal pathways or directly depressing motor nerve and muscle function (Papich 2016).

In wildlife immobilisation, the use of midazolam, in combination with ketamine or potent opioids, has gradually become more popular. This is particularly true in avian species (Ajadi et al. 2009; Araghi et al. 2016; Horowitz et al. 2014; Schaffer et al. 2017), predators (Colburn et al. 2017; Eggers 2016; Shilo et al. 2010; Wenger et al. 2010), primates (Adami & Wenker 2013; Bertrand, et al. 2016; Ishibashi 2016; Ochi et al. 2014; Wenger et al. 2013) and rhino (Van Zijll Langhout et al. 2016). However, literature is still lacking on information on the effects of midazolam, when used in combination with potent opioids, for the immobilisation of African ungulate species.

The study aimed to compare the effects of a combination of etorphine and midazolam to that of the more commonly used combination of etorphine and azaperone, for the immobilisation of blesbok.

Research methods and design

Animals and housing

The study was undertaken at the Wildlife Pharmaceuticals Research Facility, South Africa (25°31'25.2" S, 31°06'50.8" E), in March 2019. Twelve female blesbok (59.3 ± 2.8 kg) were captured from another game farm and transported to the facility two weeks before the start of the study. The blesbok were housed in groups of four in three adjacent enclosures (bomas), measuring 6 x 8 m in size. Throughout the study, feed and water was given *ad libitum*, except for 12 hours before each trial when they were withheld.

Study design

The study consisted of a blinded randomised crossover design. Each animal received each treatment once with a washout period of one week between treatments. The first treatments were allocated in a randomised manner by means of coin toss and only the lead veterinarian darting the blesbok was aware of treatment allocations. The treatments were as follows: Treatment 1 (T1): 4 mg etorphine hydrochloride [Captivon 98, 9.8 mg/ml, Wildlife Pharmaceuticals (Pty) Ltd., South Africa] + 21 mg azaperone [100 mg/ml, Wildlife Pharmaceuticals (Pty) Ltd., South Africa]; Treatment 2 (T2): 4 mg etorphine + 12 mg midazolam [50 mg/ml, Wildlife Pharmaceuticals (Pty) Ltd., South Africa]. Doses were selected based on published reports on the use of these medicines in wildlife as well as personal experience (Du Plessis 2018; Kock & Burroughs 2012). Blesbok were weighed and ear-tagged during the first immobilisation.

Immobilisation and monitoring

The blesbok were darted with a 1 ml, ¾" barbed-needle, darting system (Type 'P' slow-inject dart, Pneu-Dart Inc., PA, USA) projected from a gas-powered dart gun (X-Caliber, Pneu-Dart Inc., PA, USA) at a distance ranging from 5 to 10 m. Time from darting until blesbok showed the first clinical sign of the drug's effect on the CNS (time to first sign) and time from darting to when the animal was recumbent (time to recumbency) were recorded. A subjective induction (period from darting to recumbency) score was also allocated to each animal indicating the quality of induction based on the scoring system given in Table I.

Once an animal became recumbent, it was transported from the enclosure to a nearby shaded area where it could safely be monitored away from the remaining herd. The animal was then held in sternal recumbency with its head held in a lifted position and its nose pointing downwards, blind-folded, and earplugs inserted into the external ear canal to minimise external stimuli. All the monitoring equipment was attached within 5 minutes after the animal became recumbent so that physiological monitoring started at 5 minutes post-recumbency and lasted until 40 minutes post-recumbency. Physiological measurements (heart rate [HR], respiratory rate [f_R], end tidal carbon dioxide [$ETCO_2$], blood pressure [BP], peripheral haemoglobin oxygen saturation [SpO_2], rectal temperature [RT]) and subjective scoring of the quality of immobilisation (Table I) were recorded every 5 minutes of monitoring. An immobilisation score between 3 and 4 was considered good since animals were immobilised enough for safe monitoring and handling.

A veterinary monitor (Cardell 9500 HD Veterinary Monitor, Midmark Corporation, OH, USA) was used to assess $ETCO_2$ (mainstream method, Capnostat, Respironics, Inc., GA, USA) and f_R from a nasal endotracheal tube (ET) inserted into one nostril, and its cuff inflated to secure it in situ. HR and invasive BP were recorded by means of a portable monitor (IntraTorr, IntraVitals, United Kingdom) connected to an arterial catheter that was placed into either the auricular artery or the median artery of the metacarpus. The catheter was connected to a yellow catheter in-stopper so that blood samples could be collected easily from the same catheter without removing the IntraTorr. SpO_2 was assessed

Table I: Description of the scoring system used to categorise the quality of induction, immobilisation, and recovery (Gaudio et al. 2019 and Pfitzer et al. 2019)

Score	1	2	3	4	5
Induction	Slight ataxia followed by animal taking one or two attempts to sit and/or lie in sternal recumbency without signs of excitement or falling over during the process. A smooth transition into lateral recumbency may follow shortly after (excellent).	Moderate ataxia followed by animal taking one or two attempts to sit and/or lie in sternal recumbency. The animal may stumble during the process (good).	Severe ataxia followed by animal making numerous attempts to sit or lie down. Animal stumbles and falls on numerous occasions before becoming recumbent. Reaction to external stimuli (fair).	Severe ataxia but the animal does not become recumbent and/or the animal stumbles and falls repeatedly. Animal not approachable, requires a second dose of drugs (poor).	-
Immobilisation	Re-dosing is required to achieve recumbency. Risk of injury to the handler (limited effect).	Spontaneous motor activity, struggling during manipulation, presence of anal and palpebral reflexes, responsive to painful stimuli, might vocalise, presence of nystagmus, chewing, ear movements and strong panniculus reflex (deep sedation).	Muscle rigidity, slow palpebral reflex, voluntary tail movements, central eye position. Might vocalise, some chewing, ear movements might be absent, weak nystagmus, attenuated panniculus and anal reflex. Animal can be handled safely (light immobilisation plane).	Smooth, complete relaxation, extractable tongue, loss of palpebral reflex and jaw tone, no involuntary tail movements, ventromedial eye position, no nystagmus, no panniculus and anal reflex, no reaction to blood sampling, safe handling (deep immobilisation plane).	Too deep, absent reflexes, cardiorespiratory depression that compromises the welfare of the animal and requires reversal of immobilisation (excessively deep).
Recovery	Stands in one or two attempts and is sufficiently recovered to walk with only slight ataxia (excellent).	Some imbalance in sternal recumbency and requires more than two attempts to stand. Walks with moderate ataxia and lack of coordination (good).	Animal remains in lateral recumbency for some time following the administration of drug antagonist, is not responsive to stimuli and makes no attempt to transition to sternal recumbency. Or animal has a stormy recovery with marked ataxia and the potential for injury. May require sedation (poor).	Animal does not recover and eventually dies, or its conditions are such that it needs to be euthanised (unacceptable).	-

by means of a pulse oximeter (Nonin PalmSat 2500, Netherlands) with the reflectance probe fixed with tape to the skin under the tail. RT was measured by means of a digital thermometer (Hanna Checktemp 1, Hanna Instruments [Pty] Ltd., NE, USA). Determination of heart rate by auscultation with a stethoscope and respiratory rate by visual assessment of movement of the thorax and/or nares confirmed accuracy of veterinary monitor. Five arterial blood samples were collected anaerobically at 5, 10, 15, 20 and 30 minutes after recumbency from the auricular artery by means of a heparinised syringe. Blood gas analysis was performed using a portable analyser (EPOC Reader Blood Analysis and pre-calibrated EPOC BGEM smart cards, Epocal, Kyron Laboratories, Johannesburg, RSA). The reader was also used to measure barometric pressure at the time of sampling as well as environmental temperature. Variables measured were: arterial blood pH (pH), partial pressure of arterial oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$). All variables were measured at 37 °C. The alveolar-arterial oxygen partial pressure gradient (A-a gradient) was calculated for an open system (constant pressure) from the formula: $(A\text{-}a)O_2 = FiO_2 (Pb - PH_2O) - PaCO_2 - PaO_2$, where FiO_2 is the fractional inspired oxygen (0.209), Pb the measured barometric pressure (mmHg) and PH_2O the water vapour pressure of saturated air in the alveoli. PH_2O (mmHg) was calculated as $4.58\ e\{(17.27Tb) / (237.3 + Tb)\}$, where Tb is the body temperature (Buss et al. 2015).

At the end of the 40-minute monitoring period, the dart wound was treated, and the animal transported back to the enclosure. The animal was again placed in sternal recumbency and naltrexone (20 mg per mg etorphine) (Trexonil, 50 mg/ml, Wildlife Pharmaceuticals [Pty] Ltd., South Africa) was administered into the jugular vein to antagonise etorphine's effects. The time from naltrexone injection to the first sign of responsiveness (first sign of recovery), the time until the animal lifted its head (time to head up), the time until the animal stood up (time to standing), and the time until the animal was walking (time to walking) were recorded. Recovery period from naltrexone injection to standing was subjectively scored (Table I). All subjective scoring was done by the same trained observer (LLL) who was blinded to the treatment allocation.

Statistical analysis

For all data, mean ± standard deviation (SD) (parametric data: physiological variables, arterial blood gas analysis values, A-a gradients, induction times, and recovery times), or median (range) (nonparametric data: induction, immobilisation and recovery scores) were calculated. Physiological data, arterial blood variables, and A-a gradients were analysed using two-way ANOVA with fixed effects of time (physiological variables: 5, 10, 15, 20, 25, 30, 35 and 40 minutes; arterial blood variables and A-a gradients: 5, 10, 15, 20, 30 minutes) and treatment (T1 vs T2) with animals as repeated effect. Post-hoc pairwise comparisons were performed using a Bonferroni correction. Inter-treatment differences between induction and recovery times were analysed with a one-way ANOVA. Mann–Whitney and Kruskal–Wallis tests were used to analyse nonparametric data. Normality was verified by scatter and normality plots of standardised residuals. For all statistical analysis, Statistica 13.5.0 software was used. Any *p*-values of ≤ 0.05 were considered statistically significant.

Results

Initially, a total etorphine dose of 3 mg per blesbok was selected but after darting the first blesbok, it became apparent that this dose was too low to adequately immobilise the blesbok for safe monitoring. The data from this blesbok was subsequently excluded from the data analysis and the etorphine dose increased to 4 mg per blesbok, which provided adequate immobilisation. For the purpose of this paper, the term immobilisation will refer to a restriction of muscular activity so that an animal can be handled without resist. This is unlike the term anaesthesia which, for the purpose of this study, will refer to a state of unconsciousness that is characterised by controlled and reversible depression of the CNS and a complete loss of sensation. None of the animals in this study was observed as being completely anaesthetised, and therefore, the results are discussed in the context of immobilisation. Actual treatment doses were calculated as: T1: 0.07 ± 0.003 mg/kg etorphine + 0.36 ± 0.02 mg/kg azaperone; T2: 0.07 ± 0.003 mg/kg etorphine + 0.20 ± 0.01 mg/kg midazolam.

Mean barometric pressure was 694.4 ± 2.3 mmHg (Range: 691.1–698.0 mmHg) and mean environmental temperature was 28.5 ± 4.1 ºC (Range: 21.2–35.0 ºC) during the study. The quality of the inductions and recoveries did not differ between treatments and all the blesbok were allocated induction and recovery scores of 1. Complete immobilisation was achieved in all the blesbok, and the induction and recovery times are given in Table II. No significant differences in these times were observed between treatments.

Table II: The induction and recovery times of blesbok when darted with T1 (etorphine + azaperone) and T2 (etorphine + midazolam)

Parameter	T1	T2
Time to first sign (min)	1.38 ± 0.48	1.49 ± 0.87
Time to recumbency (min)	3.15 ± 0.70	3.53 ± 2.24
First sign of recovery (min)	0.28 ± 0.09	0.25 ± 0.07
Time to head up (min)	0.40 ± 0.15	0.42 ± 0.18
Time to standing (min)	0.76 ± 0.25	0.71 ± 0.41
Time to walking (min)	0.85 ± 0.37	0.76 ± 0.41

Clinical respiratory variables recorded over the immobilisation period in T1 and T2 are depicted in Figure 1. No differences were observed between treatments in any of these respiratory variables when taken as overall means. However, when blesbok received T2, they had lower respiratory frequencies from 25 minutes onwards compared to at 5 minutes of monitoring. $EtCO_2$ in this treatment was also higher at 35 and 40 minutes of monitoring compared to at 5 minutes.

Immobilisation scores were given subjectively and intervals of 0.5 could be given if the level of immobilisation fell between two scores. This happened, for example, when an animal was easily handled and appeared completely relaxed but still maintained some palpebral reflexes. Subsequently this animal would be scored an immobilisation score of 3.5. The mean quality of immobilisation (as indicated by the immobilisation score [IS]) was higher for blesbok when treated with T1 compared to T2. At individual time points, IS was significantly higher in blesbok when treated with T1 compared to T2 at 5 minutes of monitoring. There were no significant observed differences at any of the other time points. The quality of immobilisation improved in both treatments over time (Figure 2). This improvement only became significant from 30 minutes (T1) and 20 minutes (T2) onwards.

RT was significantly higher in blesbok treated with T2 than those treated with T1 from 10 minutes onwards throughout the monitoring period (Figure 3). The RT of blesbok when treated with T2 also increased throughout the monitoring period, but no such increase was observed when blesbok received T1 (Figure 3).

Overall, HR did not differ significantly between treatments (T1: Mean = 75 ± 28 bpm, Range = 66–91 bpm; T2: Mean = 80 ± 25 bpm, Range = 74–89 bpm). The HR of blesbok when treated with T1 was lower from 20 minutes onwards compared to at 5 minutes of monitoring. In blesbok when treated with T2, HR was lower at 35 and 40 minutes of monitoring compared to at 5 minutes of monitoring.

Blesbok, when treated with T1, had significantly lower systolic, diastolic and mean arterial pressures compared to when treated with T2. Additionally, these pressures decreased significantly from 15 minutes of monitoring onwards compared to at 5 minutes of monitoring when blesbok were treated with T1, but no such change was observed in blesbok when treated with T2 (Figure 4).

Blood gas results are given in Table III. Significant differences between treatments and over time are indicated.

Most notably, $PaCO_2$ increases from 20 minutes of monitoring (although not significantly) while PaO_2 decreases from 20 minutes of monitoring in T2. This is accompanied by a significant increase in A-a gradient. These changes are not observed for T1.

Discussion

The combination of etorphine and azaperone vs etorphine and midazolam resulted in similar induction and recovery times in

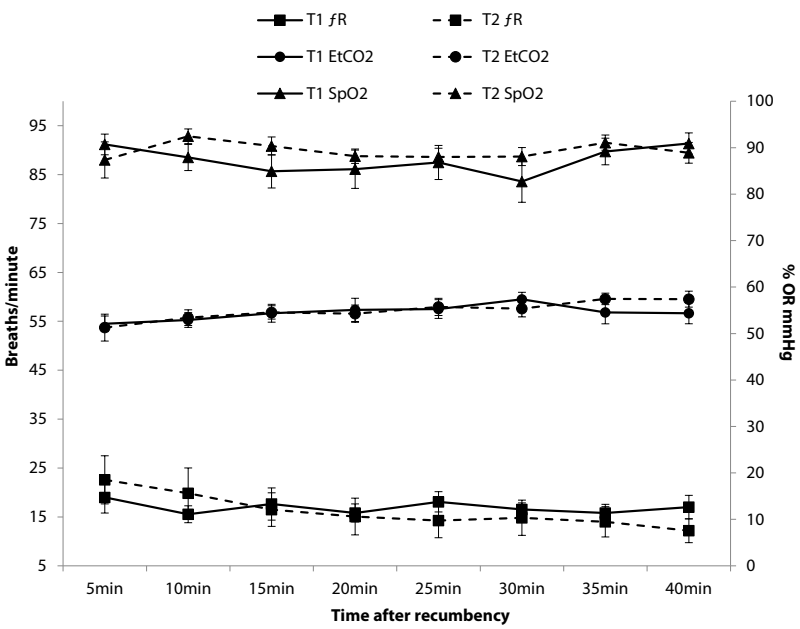

Figure 1: Clinical respiratory variables (f_R, $EtCO_2$ and SpO_2) over time in blesbok treated with T1 (etorphine + azaperone) and T2 (etorphine + midazolam)

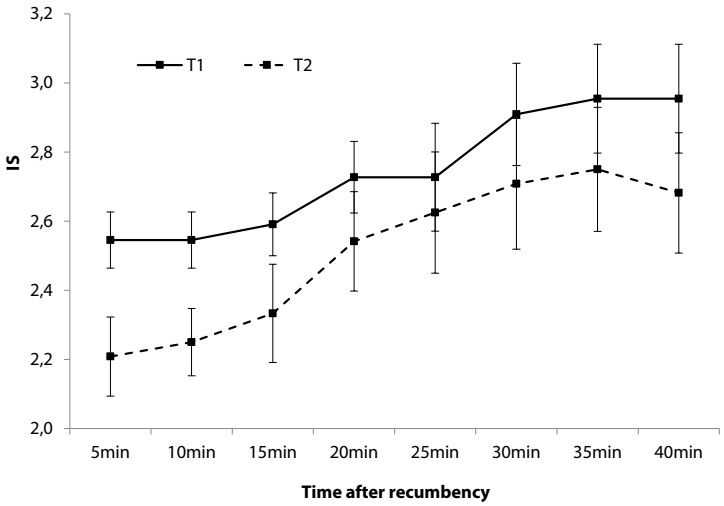

Figure 2: Changes in immobilisation scores (IS) over time in blesbok treated with T1 (etorphine + azaperone) and T2 (etorphine + midazolam)

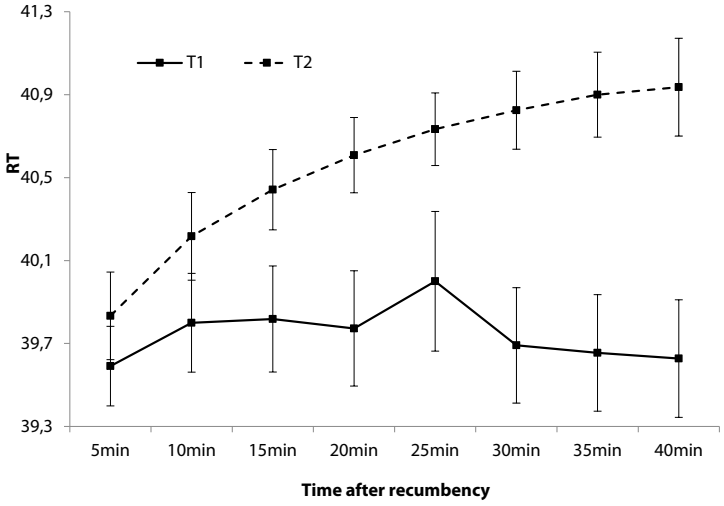

Figure 3: Changes in rectal temperature (RT, ºC) over time in blesbok treated with T1 (etorphine + azaperone) and T2 (etorphine + midazolam)

blesbok. Significant differences were, however, found in the quality of immobilisation as well as the physiological response of the blesbok to these drug combinations. The time to recumbency did not differ between the combinations (T1 = 3.2 ± 0.7 minutes; T2 = 3.5 ± 2.2 minutes) and was similar to results reported by Pfitzer et al. (2019) when 0.09 mg/kg etorphine was used as the sole drug to immobilise blesbok. It therefore appears that the addition of azaperone or midazolam to etorphine did not influence the time to recumbency. Following etorphine antagonism with naltrexone, blesbok were not ataxic or overtly sedated with either combination. Similar results were observed by Pfitzer et al. (2019) when only etorphine was used. This suggests that the addition of azaperone or midazolam at the doses used here is suitable for chemical immobilisation when animals are to be released back into the field and no residual sedation is desired.

From 20 (T2) and 30 (T1) minutes after recumbency and onwards, immobilisation quality improved in both treatments. This suggests that both midazolam and azaperone started to clinically influence the immobilisation quality around these times. In alpacas, injected intramuscularly (IM) with 0.5 mg/kg midazolam only, sedation started after 15 minutes and peaked at 23 minutes (Aarnes et al. 2013).

Similarly in sheep, time to peak sedation after IM administration of 0.5 mg/kg midazolam was reported as 22.5 minutes (Simon et al. 2017). In blesbok immobilised with a combination of etorphine and azaperone, increased CNS depression was observed at 20 minutes after recumbency (Gaudio et al. 2019). It therefore appears that the effects of both the combination of etorphine/azaperone and etorphine/midazolam, at the doses used in the current study, on CNS depression only becomes clinically apparent after 20 minutes after recumbency. Their effect is also gradual in onset. Considering the fast onset of immobilisation when etorphine is used on its own (Pfitzer et al. 2019), should an improvement in the quality of immobilisation be required, one might therefore also consider administering azaperone or midazolam intravenously as soon as the animal can be handled.

The differences in RT between the two combinations were statistically and clinically significant. While the RT of blesbok treated with T1 only increased by 0.2 °C and started decreasing within 30 minutes, RT of T2-treated blesbok started rising after immobilisation up until 40 minutes and reached nearly cytotoxic levels of 40.9 °C. In mammals, body temperatures in excess of 41 °C can result in tissue and organ damage and may be fatal above 42 °C (Fajardo 1984; Sjaastad et al. 2016). The positive effect of

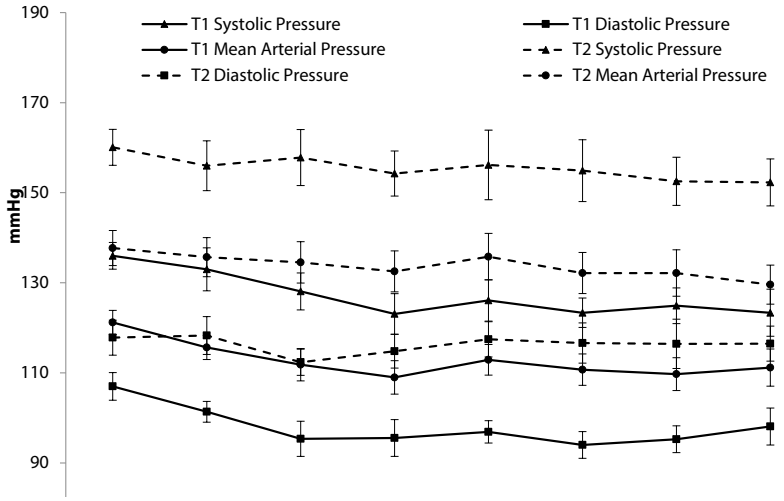

Figure 4: Changes in blood pressure over time (minutes) in blesbok immobilised with T1 (etorphine + azaperone) and T2 (etorphine + midazolam)

T1 on rectal temperature could be caused by greater heat loss from the peripheral vasodilating effects of azaperone as a result of its action on peripheral alpha$_1$-receptors (Buss et al. 2016; Kock & Burroughs 2012). The addition of azaperone to etorphine in a dart mixture could therefore have the advantage of allowing excessive heat, caused by capture-induced hyperthermia, to be lost into the environment. Additionally, this may assist in making cooling methods, such as dousing the animal with water, more efficient (Sawicka et al. 2015).

The mean heart rate of conscious blesbok at rest was reported by Du Plessis (2018) as 104 bpm. In the current study, the heart rate of blesbok when treated with T1 varied between 66 and 91 bpm (mean = 75 bbpm) while with T2, heart rate varied between 74 and 89 beats/minute (mean = 80 bpm). With both treatments, the greatest heart rate was measured at the beginning of immobilisation. Thus, blesbok had lower heart rates, compared to non-immobilised blesbok at rest, throughout the immobilisation regardless of treatment. Low heart rates caused by potent opioids in blesbok have been observed by Pfitzer et al. (2019) and Gaudio et al. (2019) and in goats by Heard, Nichols, Buss & Kollias (1996) and have been ascribed to possibly being a reflex to acute opioid-induced hypertension. Prothero (2015) describes a mean MAP of 115 mmHg as normal in most mammals, except for giraffe (*Giraffa camelopardalis*). During both treatments in the current study, blesbok suffered from hypertension at the beginning of immobilisation. However, while elevation of MAP with T1 was only moderate (121.2 ± 9 mmHg) and decreased to normal levels within 10 minutes (116 ± 9 mmHg), MAP with T2 was greater and remained elevated throughout the monitoring period (mean = 133.8 ± 15.5 mmHg). Hypertension in conjunction with opioid treatment of blesbok has also been reported by Pfitzer et al. (2019) and Gaudio et al. (2019). Gaudio et al. (2019) found that the addition of 0.35 mg/kg azaperone to 0.09 mg/kg etorphine significantly lowered MAP by about 20 mmHg over a 40-minute monitoring period when compared to treatment with just 0.09 mg/kg etorphine. In pachyderms and equids, opioid-induced hypertension has been well described and the addition of azaperone to an immobilisation mixture is not just used to provide smooth induction and an opioid-sparing effect, but also to counteract severe hypertension (Hattingh, Knox & Raath 1994; Hattingh, Knox, Raath & Keet 1994; Kock & Burroughs 2012). In contrast to the etorphine and azaperone combination, the etorphine and midazolam combination did not have the same pressor effects. While the mean MAP of 134 mmHg induced by T2 may not be considered excessively high, it needs to be considered if other drugs which may also increase blood pressure, such as ketamine or alpha-2 agonists, are to be added to this combination or used as "top-ups" during the immobilisation (Pipkin & Waldron 1983). Furthermore, field capture scenarios that can cause severe stress-induced hypertension, such as darting from a helicopter, may further amplify this hypertension and should be considered when using this combination.

Table III: Blood gas variables (mean ± SD) recorded during immobilisation of blesbok with T1 (etorphine + azaperone) and T2 (etorphine + midazolam)

Time point		pH	$PaCO_2$	PaO_2	A-a gradient
5 min	T1	7.46 ± 0.04	41.1 ± 4.9	48.8 ± 10.1	46.0 ± 2.6
	T2	7.49 ± 0.06	37.7 ± 5.6	54.7 ± 12.2	43.7 ± 2.6
10 min	T1	7.45 ± 0.04	42.8 ± 4.9	45.0 ± 11.8	48.1 ± 2.6
	T2	7.49 ± 0.05	37.1 ± 4.7	54.3 ± 10.3	44.7 ± 2.6
15 min	T1	7.45 ± 0.05	43.9 ± 6.6	43.8 ± 13.2	48.1 ± 2.6
	T2	7.49 ± 0.05	38.7 ± 5.0	48.4 ± 9.4	48.2 ± 2.6
20 min	T1	7.44 ± 0.05	45.1 ± 7.6*	41.8 ± 9.2*	48.9 ± 2.6
	T2	7.49 ± 0.05	37.9 ± 5.8	46.0 ± 8.0*	49.7 ± 2.6*
30 min	T1	7.44 ± 0.04	46.0 ± 6.3*	45.0 ± 9.3	44.8 ± 2.6
	T2	7.49 ± 0.05	41.2 ± 6.5	44.3 ± 9.4*	49.8 ± 2.6*
Overall	T1	7.45 ± 0.04	43.7 ± 6.1	44.8 ± 10.6	47.3 ± 8.5
	T2	7.49 ± 0.05	38.8 ± 5.5	49.7 ± 10.5	47.5 ± 9.4

PaO_2 – partial pressure of oxygen, $PaCO_2$ – partial pressure of carbon dioxide, A-a gradient – alveolar-arterial oxygen partial pressure gradient
Significant inter-treatment differences at the same time point ($p < 0.05$) are highlighted in greyscale.
* Indicates values that are significantly different ($p < 0.05$) from values at 5 minutes.

The respiratory rate of conscious blesbok lying down to rest has been reported to be 13 breaths/minute while standing blesbok had a respiratory rate of 20 breaths/minute (Du Plessis 2018). Therefore, the overall respiratory rates recorded during immobilisation with both T1 (16.9 ± 7.4 breaths/minute) and T2 (16.2 ± 13.2 breaths/minute) were within physiological range. However, hypoxaemia was pronounced during both treatments, although more severe with T1 (overall mean PaO_2 = 44.8 ± 10.6 mmHg) than with T2 (overall mean PaO_2 = 49.7 ± 10.5 mmHg). The elevated A-a gradients also indicated that both drug combinations caused impairment of alveolar gas exchange, resulting in an oxygen diffusion deficit. An oxygen diffusion deficit could stem from various drug-induced changes such as pulmonary congestion or oedema, caused by pulmonary hypertension, red blood cells traversing too fast through alveoli capillaries to adequately saturate haemoglobin, shunting as well as ventilation-perfusion mismatching (Meyer et al. 2015). Azaperone on its own is not believed to have significant effects on respiration (Radcliffe, Ferrell & Childs 2012). However, when combined with etorphine, compromised pulmonary gas exchange in blesbok has also been reported by Gaudio et al. (2019). Interestingly, the two treatments exhibited different changes in PaO_2 over time, particularly from 20 minutes of monitoring. While $PaCO_2$ increased and PaO_2 decreased after 20 minutes with T2, PaO_2 increased with T1 from 20 minutes onwards. Consequently, the highest A-a gradients were recorded during T2 at 30 minutes of monitoring. Respiration rate also decreased over time in the animals that received T2 whereas those that received T1 appeared to have a much more stable respiration rate. When taking into consideration that the quality of immobilisation improved over time with both treatments, it could be that this improvement, especially with T2, may have had detrimental effects on respiration. In humans, midazolam has been associated with respiratory depression. It has been postulated that this is as a result of direct depression of the central respiratory drive as well as simultaneous depression of respiratory muscle efficiency (Gross, Smith & Tranquilli 1993). Similar results have been reported in cats (Gross et al. 1993) and goats (Stegmann 1999). In goats, Stegmann (1999) postulated that the muscle relaxation induced by midazolam may reduce ventilatory muscle function, contributing towards a reduction in tidal volume and an increase in hypoxaemia. The increase in immobilisation quality observed in the current study may, in fact, have been a clinical observation of increased muscle relaxation. Therefore, it stands to reason that this may well have been what contributed to the deterioration of respiratory efficacy, resulting in inadequate oxygen uptake so that an increase in A-a gradient was observed towards the end of the monitoring period with T2. However, to understand how this inadequate oxygen uptake occurred would require an in-depth assessment of the interaction and function of the cardiovascular and pulmonary systems.

Despite the paucity of literature on the use of midazolam in antelope, some doses have been published. In Nile lechwe (*Kobus magaceros)*, 0.31 mg/kg midazolam was used in combination with 0.2 mg/kg butorphanol and 0.2 mg/kg detomidine (Laricchiuta et al. 2012). This combination was effective in immobilising captive healthy lechwes with minimal cardiorespiratory changes. In Nubian ibex (*Capra nubiana*), the combination of 0.13 mg/kg butorphanol, 0.13 mg/kg midazolam, and 0.13 mg/kg medetomidine was reported to be effective for short-term immobilisation. Du Plessis (2018) reported that when given on its own as a sedative after immobilisation, a dose of 0.2 mg/kg midazolam was most effective in sedating blesbok. The author found that doses of 0.6 mg/kg midazolam and higher resulted in the occurrence of extrapyramidal effects and severe ataxia (*personal experience*). Although it may be that the midazolam doses used in the current study were not adequate enough to result in the same immobilisation quality as observed when azaperone was used, it may also be that the combination of etorphine and midazolam is merely not as effective as the combination of etorphine and azaperone. Furthermore, an increased midazolam dose may have increased midazolam's effect on respiration which could have exacerbated opioid-induced respiratory depression.

Limitations of this study

The inclusion of a third treatment of etorphine alone, at the dose used in this study, would have allowed the comparison of the individual effects of azaperone vs midazolam on immobilisation. A more thorough cardiopulmonary assessment would be required to see how both ventilation and gas diffusion/exchange differed between the treatments. Furthermore, measuring metabolism would have also helped to understand the blood gases, and effects of the drugs better. With the lack of research available on the effective dose of midazolam to use in ungulate species, the inclusion of an additional treatment with a higher dose of midazolam could potentially have provided valuable information on what the effect of midazolam is in blesbok and specifically whether a higher dose would result in better immobilisation but possibly worsen respiration.

Recommendations for future research

Future research could possibly investigate the dose effect of midazolam in combination with potent opioids. The inclusion of a treatment consisting of only a potent opioid would also allow researchers to elucidate on the exact contribution of a specific sedative or tranquiliser to the physiological response of animals to an immobilisation drug combination.

Conclusion

In summary, both treatments resulted in the adequate immobilisation of blesbok with the quality of immobilisation improving over time. The combination of etorphine and midazolam resulted in hyperthermia and more pronounced respiratory compromise towards the end of the monitoring period. The combination of etorphine and azaperone resulted in a subjectively deeper plane of immobilisation, better temperature regulation and less severe systemic hypertension. These latter effects are likely the result of the peripheral $alpha_1$-mediated vasodilating effects of azaperone and can be considered a positive outcome, specifically under field conditions where chemical capture techniques can result in stress-induced hyperthermia and hypertension. However, blesbok immobilised with the combination of etorphine and azaperone also suffered from more pronounced hypoxia so

that both treatments negatively affected respiration. The choice of combination should therefore be based on how expected capture-induced effects will interact with the drug effects to achieve the optimal physiological stability during immobilisation. Since both combinations produced clinical hypoxaemia, oxygen supplementation is recommended especially if prolonged immobilisation is required.

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Conflict of interest

JP Raath, one of the investigators, has a financial interest in Wildlife Pharmaceuticals Pty Ltd., the company which also supported this project financially. However, we do not believe that this would have inappropriately influenced the analysis or writing of this article.

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Ethical approval

The study was approved by the Wildlife Pharmaceuticals Animals Ethics Committee (Approval Number: WPAEC-2018-ZAPDAZBLES-33-B).

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