RESEARCH PAPER

Immobilization quality and cardiopulmonary effects of etorphine alone compared with etorphine–azaperone in blesbok (*Damaliscus pygargus phillipsi*)

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Abstract

Objective To evaluate the immobilization quality and cardiopulmonary effects of etorphine alone compared with etorphine–azaperone in blesbok (*Damaliscus pygargus phillipsi*).

Study design Blinded, randomized, crossover design.

Animals A total of 12 boma-habituated female blesbok weighing [mean ± standard deviation (SD)] 57.5 ± 2.5 kg.

Methods Each animal was administered etorphine (0.09 mg kg^{-1}) or etorphine–azaperone (0.09 mg kg^{-1}; 0.35 mg kg^{-1}) intramuscularly with 1-week intertreatment washout period. Time to first sign of altered state of consciousness and immobilization time were recorded. Physiological variables were recorded, arterial blood samples were taken during a 40-minute immobilization period, and naltrexone (mean ± SD: 1.83 ± 0.06 mg kg^{-1}) was intravenously administered. Recovery times were documented, and induction, immobilization and recovery were subjectively scored. Statistical analyses were performed; $p < 0.05$ was significant.

Results No difference was observed in time to first sign, immobilization time and recovery times between treatments. Time to head up was longer with etorphine–azaperone (0.5 ± 0.2 *versus* 0.4 ± 0.2 minutes; $p = 0.015$). Etorphine caused higher arterial blood pressures (mean: 131 ± 17 *versus* 110 ± 11 mmHg, $p < 0.0001$), pH, rectal temperature and arterial oxygen partial pressure (59.2 ± 7.7 *versus* 42.2 ± 9.8 mmHg), but lower heart ($p = 0.002$) and respiratory rates ($p = 0.01$). Etorphine–azaperone combination led to greater impairment of ventilatory function, with higher end-tidal carbon dioxide ($p < 0.0001$) and arterial partial pressure of carbon dioxide (58.0 ± 4.5 *versus* 48.1 ± 5.1 mmHg). Immobilization quality was greater with etorphine-azaperone than with etorphine alone (median scores: 4 *versus* 3; $p < 0.0001$).

Conclusions and clinical relevance Both treatments provided satisfactory immobilization of blesbok; however, in addition to a deeper level of immobilization, etorphine–azaperone caused greater ventilatory impairment. Oxygen supplementation is recommended with both treatments.

Keywords azaperone, blesbok, cardiopulmonary function, etorphine, quality of immobilization.

Introduction

Chemical immobilization is described as the use of chemical agents to restrict the movement of animals, thus enabling them to be approached safely and restrained to allow their examination. Chemical immobilization is frequently used across Southern Africa in antelope species such as blesbok (*Damaliscus pygargus phillipsi*) for medical or management purposes or both. Drug combinations used for the immobilization of blesbok generally include opioid-based drug combinations (Kock & Burroughs 2012).

Etorphine is a potent opioid that has been widely used in the past for chemical immobilization of wild African herbivores (Kock & Burroughs 2012). Commonly reported side effects associated with the use of etorphine include hyperthermia, ventilatory depression, bradycardia, tachycardia, hypertension and muscle excitation (Grimm et al 2015). In order to reduce the opioid dose and improve the quality of immobilization, opioids are often co-administered with an α_2-adrenoceptor agonists or butyrophenones such as azaperone (Kock & Burroughs 2012).

Azaperone is a neuroleptic drug with tranquilizing effects that are usually observed 10–20 minutes after intramuscular (IM) injection with a duration of effect that lasts up to 6 hours in horses (Lees & Serrano 1976). Butyrophenones act mainly *via* dopaminergic (D_2) antagonism, resulting in tranquilization and potentiation of immobilization, especially when used in combination with potent opioids (Swan 1993; Riviere & Papich 2009; Kock & Burroughs 2012). They also have a mild antagonistic action on α_1-adrenoceptors, causing peripheral vasodilation and bradycardia (Lees & Serrano 1976). These effects are considered to be beneficial during wildlife capture as they reduce the hypertensive effects of potent opioids and α_2-adenoceptor agonists (Meyer et al 2008). Furthermore, azaperone also exerts its tranquilizing effect after the other drugs have been antagonized, which is beneficial during the translocation of wildlife (Still et al 1996; Kock & Burroughs 2012; Zeiler & Meyer 2017a).

Currently, there are limited reports on the use of azaperone in African antelope species (Meyer et al 2008; Zeiler & Meyer 2017a; Semjonov et al 2018). Its effects on cardiovascular function have previously been studied in the horse, pig, rhinoceros and African elephant (Lees & Serrano 1976; Still et al 1996; Buss et al 2016). Furthermore, no reports of its effects on ventilatory function are available; however, some authors suggest that it may improve ventilation during wildlife immobilization (Haigh 1990; Swan 1993; Radcliffe et al 2012). Preliminary work by the current authors has found that azaperone may accentuate the ventilatory depressant effects of potent opioids in wild antelope species.

The present study aimed to investigate the cardiopulmonary effects and quality of immobilization of etorphine alone and etorphine–azaperone combination in blesbok. It was hypothesized that the latter treatment would cause a greater impairment of ventilation and a deeper plane of immobilization than etorphine alone.

Materials and methods

Animals and housing

The study was undertaken at the Wildlife Pharmaceuticals Research Facility, in March 2018 and approved by the Wildlife Pharmaceuticals Animals Ethics Committee (Approval number: WPAEC-2018-AZAPBLES-25-B). A total of 12 wild blesbok (all female) were acquired from a game farm and transported to the facility 2 weeks before the start of the study. Following a 1 week acclimatization period at the facility, all animals were darted for a preliminary health check comprising physical examination, complete blood count, blood biochemistry test, weighing and ear tagging. The animals were housed in groups of four in three adjacent enclosures (bomas), measuring 6 × 8 m in size. During the acclimatization period, feed and water was given *ad libitum*.

Study design

The research was conducted in the form of a blinded randomized crossover study. Sample size calculation was performed analysing the means and standard deviations of the paired differences of cardioventilatory variables [e.g. arterial partial pressure of oxygen (PaO_2), arterial partial pressure of carbon dioxide ($PaCO_2$) and alveolar-arterial oxygen partial pressure gradient (A-a gradient)] from previous research (Meyer et al 2015) using SAS statistical software Version 9.3 (SAS Institute, NC, USA). Power analysis was performed by specifying $\alpha = 0.05$ and power = 0.8. With an effect size of 1.63 for PaO_2, 1.61 for $PaCO_2$ and 1.23 for A-a gradient, it was determined that a minimum of 8, 8 and 10 animals would be required, respectively. In order to reduce the bias further, it was decided to use 12 animals in this study. Each animal was administered each drug or drug combination once with a 1 week washout period between treatments. Randomization was achieved *via* a simple random sampling method by means of SAS Version 9.3 statistical software (SAS Institute, NC, USA) and assessors were blinded to treatment allocation. The treatments were as follows: treatment 1 (T1E): etorphine, 0.09 mg kg^{-1} [Captivon 98, Wildlife Pharmaceuticals (Pty) Ltd, South Africa]; treatment 2 (T2EA): etorphine 0.09 mg kg^{-1} and azaperone 0.35 mg kg^{-1} [Zapnil, Wildlife Pharmaceuticals (Pty) Ltd]. The individual dose was calculated on the basis of the bodyweight of the animals measured during the acclimatization period.

Immobilization and monitoring

Water and feed were withheld for 12 hours prior to the start of each trial. The drugs were withdrawn from their vials using 1 mL syringes and subsequently injected and mixed together within the dart, which was ultimately filled up with sterile sodium chloride solution (Vetivex Veterinary 0.9% Sodium Chloride Injection, USP, Dechra Veterinary Products Ltd, UK). The animals were darted with a 1.5 mL, 1.91 cm barbed needle darting system (Type 'P' RDD Device, Pneu-Dart. Inc, PA, USA) projected from a gas-powered dart gun (X-Caliber, Pneu-Dart. PA, USA) at a distance ranging from 5 to 10 m. Time from darting to first sign of altered state of consciousness (e.g.

ataxia and altered proprioception; time to first sign) (Kock & Burroughs 2012) and time from darting to recumbency (immobilization time) were recorded. A subjective score (induction score) for the induction phase, deemed as the period from drug injection to immobilization, was also documented (Table 1) (Wenger et al. 2010).

After recumbency, the animal was transported using a stretcher from the boma to a nearby shaded area, which had been prepared in advance for monitoring. The animal was positioned in sternal recumbency on a table and supported by a handler who held the horns so that the neck was aligned with the vertebral column and the head was elevated above the thorax with the nose pointing downward. The blesbok was immediately blindfolded and earplugs were inserted into the external ear canal. An endotracheal tube (premeasured to extend from the caudal end of the nasal bone to the rostral part of the frontal bone) was inserted into one nostril, advanced to the level of the nasopharynx, and its cuff inflated to secure it *in situ* for assessment of ventilatory variables. An arterial catheter was placed into the caudal auricular artery or, alternatively, the median artery of the metacarpus. Evaluation of physiological variables and assessment of the quality of immobilization were performed at 5 minute intervals starting at 5 minutes after recumbency for a duration of 40 minutes (Table 1).

A multiparameter monitor (Cardell 9500 HD Veterinary Monitor, Midmark Corporation, Dayton, OH, USA) was used to measure end-tidal carbon dioxide ($P_{E'}CO_2$) (mainstream method, Capnostat, Respironics, Inc, CT, USA) and respiratory rate (f_R), whereas heart rate (HR) and invasive blood pressure (IBP) were measured using a portable IBP monitor (IntraTorr, IntraVitals, UK). Peripheral oxygen saturation of haemoglobin (SpO_2) was measured using a pulse oximeter (Nonin PalmSat 2500, The Netherlands) with the probe attached to the skin under the tail. Rectal temperature (RT) was measured using a thermometer [Hanna Checktemp 1, Hanna Instruments (Pty) Ltd, RSA]. Cardiac auscultation allowed measurement of HR, and manual counts of f_R were additionally performed to confirm the accuracy of electronic monitors. Arterial blood samples were collected anaerobically at 5, 10, 15, 20 and 30 minutes after recumbency from the caudal auricular artery using a heparinized syringe (Pro-Vent Plus 1 mL, Smiths Medical, UK) equipped with a 23 gauge needle. Blood gas analysis was performed using a portable analyser and cartridges (EPOC Reader Blood Analysis and EPOC BGEM smart cards, Epocal, Kyron Laboratories, RSA). Variables measured and corrected to body temperature by the portable analyser, were arterial blood pH (pH), base excess (BE), bicarbonate (HCO_3^-), PaO_2, $PaCO_2$, haematocrit (Hct), haemoglobin (Hb) and lactate (Lac). The A-a gradient was calculated from the formula (Sarkar et al 2017):

$$P\,(A\text{-}a)O_2 = F_IO_2\,(PB - PH_2O) - PaCO_2/RQ - PaO_2,$$

where F_IO_2 is the fractional inspired oxygen [0.209 from Sarkar et al (2017)], PB the barometric pressure (mmHg), PH_2O the water vapour pressure of saturated air in the alveoli (mmHg) and RQ the respiratory quotient. PB was measured with a calibrated portable barometer at the beginning of each immobilization (Model CPG2300,

Table 1 Description of the scoring system used to categorize the quality of induction, immobilization and recovery (modified from Wenger et al 2010) - with permission. © Association of Veterinary Anaesthetists and American College of Veterinary Anesthesia and Analgesia. Published by Elsevier Ltd.

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)	–
Immobilization	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 vocalize, presence of nystagmus, chewing, ear movements and strong panniculus reflex (deep sedation)	Muscle rigidity, slow palpebral reflex, voluntary tail movements, central eye position. Might vocalize, some chewing, ear movements might be absent, weak nystagmus, attenuated panniculus and anal reflex. Animal can be handled safely (light immobilization 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 immobilization plane)	Too deep, absent reflexes, cardiorespiratory depression (excessively deep)
Recovery	Stands in one or two attempts and is sufficiently recovered to walk with only slight ataxia. Recovery to walking occurs within 2 minutes following administration of drug antagonist (excellent)	Some imbalance in sternal recumbency and requires more than two attempts to stand. Walks with moderate ataxia and lack of coordination. Recovery to walking occurs within 5 minutes following administration of drug antagonist (good)	Animal remains in lateral recumbency for more than 5 minutes 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 with 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 euthanized (unacceptable)	–

Mensor Corporation, TX, USA). The PH_2O in the alveoli and RQ were used at constant values of 47 mmHg (Christie & Loomis 1932) and 1 (standard RQ for healthy ruminants) (Kim et al 2013), respectively.

At the end of the 40 minute immobilization phase, the overall quality of immobilization was recorded using a scoring system *ad hoc* designed, adapted from Wenger et al 2010 (immobilization score), the dart wound was treated and the animal transported back to the boma. The animal was again placed in sternal recumbency and naltrexone [mean ± standard deviation (SD): 1.83 ± 0.06 mg kg^{-1}] [Trexonil, Wildlife Pharmaceuticals (Pty) Ltd, RSA] was administered into the jugular vein *via* percutaneous administration to antagonize the effects of etorphine. Recovery times were recorded as follows: first sign of recovery, the time from naltrexone injection to the first sign of the animal becoming responsive; time to head up, the time until the animal lifted its head; time to standing, the time until the animal stood; and time to walking, when the animal was able to walk. Recovery phase (from naltrexone administration to the animal being fully conscious) was subjectively scored (Table 1). All subjective scores were assigned by the same trained observer (EG) who was masked to treatment allocation. All animals were monitored for symptoms of postanaesthetic morbidity (e.g. renarcotization or extrapyramidal signs such as dystonic reactions and excitation) twice a day for the following 15 days.

Statistical analysis

For all data, mean ± SD (parametric data: physiological variables, all measurements derived from arterial blood gas samples, A-a gradients, induction times and recovery times), or median (range) (nonparametric data: induction, immobilization and recovery score) were calculated. Data were tested for distribution type using the Shapiro–Wilk test, and non-normally distributed data were log-transformed (lactate). Physiological data, arterial blood variables and A-a gradients were analysed using a two-way analysis of variance (ANOVA) with fixed effects of time (physiological variables: 5, 10, 15, 20, 25, 30, 35 and 40 minutes; arterial blood measurements and A-a gradients: 5, 10, 15, 20, 30 minutes) and treatment (T1E *versus* T2EA) with animals as repeated effect. *Post hoc* pairwise comparisons were performed using Bonferroni correction. Intertreatment differences between induction and recovery times were analysed with a one-way ANOVA. The hypotheses (normality, identical and independent distribution) of the linear model on the residuals were graphically assessed. Mann–Whitney *U* and Kruskal–Wallis tests were used to analyse nonparametric data. Pearson's correlation was performed between RT and time to first sign and time to final recumbency. Data analyses were performed using SAS statistical software Version 9.3 (SAS Institute, NC, USA). Values of $p < 0.05$ were deemed significant.

Results

All animals were considered to be healthy based on clinical evaluations and blood tests performed during the acclimatization period. The mean weight of the animals was 57.5 ± 2.5 kg during the study period.

Difference between treatments was not observed regarding both time to first sign and immobilization time. Complete immobilization was achieved in all animals, and both T1E and T2EA had a median induction score of 1 (Table 2). In both treatments, the first signs of drug effects included ataxia and drowsiness, followed by the animal becoming sternally recumbent. Median immobilization scores are shown in Tables 2 and 3. Animals given T2EA had a significantly higher score than those given T1E score ($p < 0.0001$). The difference in immobilization score between treatments ($p = 0.003$) was apparent at 10 minutes and continued until the end of the immobilization period. Intratreatment changes were not documented in T1E over time, whereas within T2EA immobilization scores peaked at 20 minutes ($p = 0.025$). There was no significant difference between groups in first sign of recovery or time to standing. Head up time was longer in T2EA than in T1E ($p = 0.015$). Standing and walking times were simultaneous in all animals. Recovery was scored 1 in all animals (Table 2). Mortality and postanaesthetic morbidity were not evident with either treatment.

Physiological variables recorded during immobilization in T1E and T2EA are presented in Table 3. The f_R ($p = 0.01$) and $P_E'CO_2$ ($p < 0.0001$) values were greater with T2EA than with T1E. f_R decreased significantly from baseline with both T1E and T2EA at 35 ($p = 0.01$) and 40 minutes ($p = 0.03$), respectively.

Table 2 Mean ± standard deviation of induction and recovery times and median score (range) of quality of induction, immobilization and recovery of 12 female blesbok administered etorphine alone (0.09 mg kg^{-1}; T1E) or an etorphine–azaperone combination (0.09 mg kg^{-1} and 0.35 mg kg^{-1}; T2EA) intramuscularly. At the end of the 40 minute immobilization period, naltrexone (1.86 ± 0.06 mg kg^{-1}) was administered intravenously to antagonize etorphine.

Times and scores	Treatment	
	T1E	T2EA
Time to first sign (minutes)	1.4 ± 0.2	1.8 ± 0.8
Immobilization time (minutes)	2.5 ± 0.4	3.2 ± 1.9
First signs of recovery (minutes)	0.3 ± 0.1	0.4 ± 0.1
Time to head up (minutes)	0.4 ± 0.2*	0.5 ± 0.2*
Time to standing (minutes)	0.9 ± 0.4	1.2 ± 0.3
Time to walking (minutes)	0.9 ± 0.4	1.2 ± 0.3
Induction score	1 (1–2)	1 (1–2)
Immobilization score	3 (2–4)*	4 (3–4)*
Recovery score	1 (1–1)	1 (1–1)

* Indicates significant difference between treatments. The *p* values are deemed significant for values ≤0.05.

Table 3 Mean ± standard deviation of physiological variables: respiratory rate (f_R), haemoglobin oxygen saturation (SpO_2), end-tidal carbon dioxide ($P_E'CO_2$) and rectal temperature (RT) and median score (range) of quality of immobilization recorded during immobilization of 12 adult female blesbok administered etorphine alone (T1E) or etorphine–azaperone combination (T2EA). In the mean value column, the mean value of each variable calculated over the entire postrecumbency monitoring period is displayed. Refer to Table 2 legend for detailed information of drug dosages and routes of administration.

Variable	Postrecumbency time point (minutes)																	
	5		10		15		20		25		30		35		40		Mean value	
	T1E	T2EA	T1E	T2EA	T1E	T2EA	T1E	T2EA	T1E	T2EA	T1E	T2EA	T1E	T2EA	T1E	T2EA	T1E	T2EA
f_R (breaths minute^{-1})	17 ± 5	19 ± 4	15 ± 5	17 ± 3	12 ± 5	16 ± 5	13 ± 4	15 ± 5	13 ± 5	14 ± 3	13 ± 4	15 ± 3	11 ± 4*	14 ± 5	14 ± 6	13 ± 4*	13 ± 5†	15 ± 4
SpO_2 (%)	96 ± 5†	87 ± 8	96 ± 4	89 ± 5	96 ± 3	92 ± 7	96 ± 4	89 ± 9	97 ± 3	94 ± 7	97 ± 3	94 ± 6	96 ± 4	91 ± 5	96 ± 4	94 ± 6	96 ± 4†	91 ± 7
$P_E'CO_2$ (mmHg) (kPa)	57 ± 6	57 ± 6	58 ± 4	61 ± 5	59 ± 5	62 ± 4	58 ± 4	64 ± 5*	59 ± 5	62 ± 6	58 ± 4	63 ± 4*	57 ± 4†	63 ± 4*	55 ± 3†	62 ± 4	59 ± 4†	62 ± 5
	7.6 ± 0.8	7.6 ± 0.8	7.7 ± 0.5	8.1 ± 0.7	7.7 ± 0.7	8.3 ± 0.5	7.7 ± 0.5	8.5 ± 0.7	7.7 ± 0.7	8.3 ± 0.8	7.7 ± 0.5	8.4 ± 0.5	7.6 ± 0.5	8.4 ± 0.5	7.3 ± 0.4	8.3 ± 0.5	7.9 ± 0.5	8.3 ± 0.7
RT (°C)	39.2 ± 0.6	39 ± 0.4	39.4 ± 0.7	39 ± 0.4	39.3 ± 0.8	38.9 ± 0.5	39.3 ± 1.0	38.8 ± 0.5	39.4 ± 1.0	38.8 ± 0.6	39.4 ± 1.1	38.8 ± 0.6	39.4 ± 1.1†	38.7 ± 0.6	39.4 ± 1.1†	38.6 ± 0.6	39.1 ± 0.9†	38.8 ± 0.5
Immobilization score	3 (2–3)	3 (3–4)	3 (2–4)†	4 (3–4)	3 (2–4)†	4 (3–4)	3 (3–4)†	4 (3–4)*	3 (2–4)†	4 (3–4)†	3 (2–4)	4 (3–4)†	3 (2–4)†	4 (3–4)	3 (2–4)†	4 (3–4)	3 (2–4)‡	4 (3–4)

* Indicates values that are significantly different ($p < 0.05$) from values at 5 minutes.
† Intertreatment significant differences at the same time point ($p < 0.05$).
‡ Indicates significant intertreatment differences between mean values ($p < 0.05$).

$P_E'CO_2$ values did not vary over time with T1E but increased between 5 and 20 minutes with T2EA ($p = 0.005$). However, the intertreatment difference for the latter was not deemed to be clinically significant. Overall, mean SpO_2 and RT values were higher in T1E than in T2EA ($p < 0.0001$). Correlation was not observed between mean RT and time to first sign and immobilization time ($r = -0.079$; $r = -0.034$).

Intertreatment differences in systolic (SAP), diastolic (DAP) and mean arterial pressure (MAP) and HR values are displayed in Figure 1a, b, c and d, respectively. SAP, DAP, and MAP were higher in T1E than in T2EA ($p < 0.0001$). These values did not significantly change over time with either treatment. Overall mean HR ($p = 0.02$) values were greater during T2EA than during T1E (52 ± 2 *versus* 45 ± 1 beats minute^{-1}). HR did not change significantly over time with either T1E or T2EA (Figure 1d).

Arterial blood gas values, Hct, Hb, and Lac measured with T1E and T2EA are presented in Table 4. Animals treated with T1E exhibited higher PaO_2, pH ($p < 0.0001$), Hct ($p = 0.002$) and Hb ($p = 0.0002$) than those treated with T2EA. pH and PaO_2 did not change over time with either T1E or T2EA. Hct decreased at 15 minutes ($p = 0.003$) from initial values in T1E and did not vary over time in T2EA. Hb showed a similar trend as Hct, with a significant decrease at 15 minutes from baseline in T1E and did not vary over time in T2EA. Conversely, animals treated with T2EA showed higher ($p < 0.0001$) $PaCO_2$, BE and HCO_3^- values. $PaCO_2$ did not change over time during immobilization with T1E or T2EA. Intratreatment differences from values at 5 minutes were not documented with regards to BE and HCO_3^- with either treatment. Intertreatment difference was not observed in Lac, which decreased significantly at 30 minutes ($p = 0.01$) from values at 5 minutes in T1E and did not change over time in T2EA.

The mean A-a gradient was higher with T2EA than with T1E ($p < 0.0001$), and the values were significantly lower at 30 minutes ($p = 0.04$) than at 5 minutes in T2EA. Significant changes over time were not recorded in the A-a gradient values in T1E (Figure 2).

Discussion

The present study is the first to evaluate the quality of immobilization and the cardiopulmonary effects of two etorphine-based immobilization protocols in blesbok. Both treatments produced adequate, but qualitatively different, immobilization of the animals for 40 minutes.

There are no reports regarding the onset of action of azaperone in blesbok; however, in horses its onset of action occurs within 10–20 minutes following IM administration (Lees & Serrano 1976). If azaperone had a similar onset of action in blesbok, the absence of intertreatment differences in time to first sign, immobilization time and induction score may be

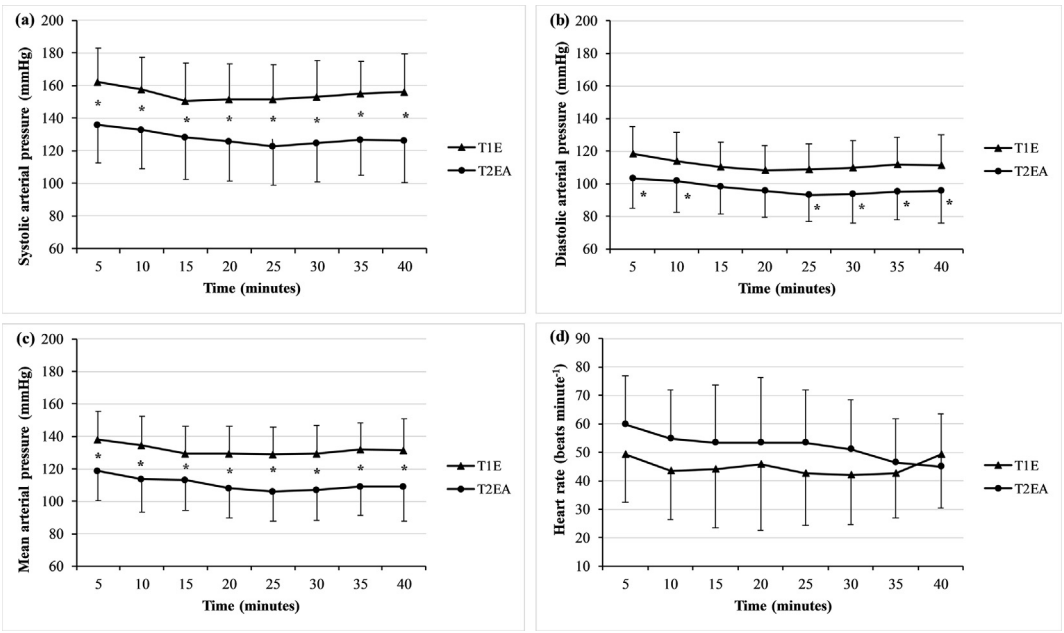

Figure 1 Mean values ± standard deviation of systolic (graph a), diastolic (graph b) and mean (graph c) arterial blood pressures, and heart rate (graph d) obtained during immobilization of 12 adult female blesbok administered etorphine alone (0.09 mg kg^{-1}; T1E) or an etorphine–azaperone combination (0.09 mg kg^{-1} and 0.35 mg kg^{-1}; T2EA) intramuscularly. For detailed legend, see Table 2. Data were recorded at 5 minute intervals once the animals were recumbent. *Intertreatment significant differences ($p < 0.05$).

explained by the initial rapid action of etorphine (Haigh 1990; Meyer et al 2008).

In the present study, blesbok were considered to be hypertensive (hypertension in conscious sheep: MAP > 95 mmHg) (Thatcher & Keith 1986) following administration of both treatments, but this complication was more marked with T1E than with T2EA (MAP: 132 ± 17 *versus* 111 ± 11 mmHg, respectively). Etorphine reportedly causes hypertension in wild and domestic animals (Kock & Burroughs 2012; Meyer et al 2015; Zeiler & Meyer 2017a,b), but the mechanisms behind this effect still require clarification. However, etorphine may either directly activate the sympathetic nervous system (Roquebert & Delgoulet 1988) or indirectly cause hypertension owing to its hypoxaemic and hypercapnic side effects (Heard et al 1990). The lower MAP values recorded with T2EA probably result from the peripheral antiadrenergic effects of azaperone (Lees & Serrano 1976) causing vasodilation and partial counteraction of this side effect of etorphine (Swan 1993; Meyer et al 2008).

Bradycardia (HR < 55 beats minute^{-1}) (Semjonov et al 2018) was documented with both treatments, but it was more pronounced with T1E (45 ± 18 and 52 ± 15 beats minute^{-1}). Opioids, such as etorphine, decrease HR as a result of opioid-induced medullary vagal stimulation (Bowdle 1998). The bradycardia documented with both treatments probably resulted from a baroreceptor-mediated reflex secondary to the etorphine-related increase in arterial blood pressure. Therefore, the higher HR recorded following T2EA administration may be explained by the lower MAP associated with azaperone administration (Lees & Serrano 1976; Mentaberre et al 2010). An alternative explanation for the higher HR documented with T2EA may relate to the degree of hypoxaemia observed, which may have led to greater sympathetic stimulation of the heart (Schultz et al 2007). This finding is notable as azaperone has been used in the past to partially counteract the respiratory depressant effects of opioids and other anaesthetic agents, thereby improving ventilation (Haigh 1990; Swan 1993).

In the present study, the combined effect of etorphine and azaperone was associated with a higher mean f_R compared with etorphine alone. The higher f_R may result from activation of central and peripheral chemoreceptors in response to the higher $PaCO_2$ recorded with T2EA (Guyenet 2014). However, this increase in f_R did not provide an overall improvement in alveolar ventilation.

Drug-induced hypoventilation was the likely cause of the hypercapnia recorded with both treatments. When breathing room air, hypoventilation may cause a reduction in PaO_2, the latter usually being responsive to oxygen administration. Hypoventilation does not cause an A-a gradient increase *per se*; however, it may rapidly lead to atelectasis (within 20 minutes in anaesthetized horses) of some parts of the lungs, thus impairing gas exchange and subsequently widening the A-a

Table 4 Mean ± standard deviation of arterial partial pressure of oxygen (PaO_2), arterial partial pressure of carbon dioxide ($PaCO_2$), haematocrit (Hct), haemoglobin (Hb), base excess (BE), bicarbonate (HCO_3^-) and lactate (Lac) values obtained from arterial blood samples withdrawn during immobilisation of 12 adult female blesbok administered etorphine alone or (T1E) and etorphine–azaperone combination (T2EA). In the mean value column, the mean value of each variable calculated over the entire postrecumbency monitoring period is displayed. For detailed legend, see Table 2.

Variable	Postrecumbency time (minutes)										Mean value	
	5		10		15		20		30			
	T1E	T2EA	T1E	T2EA	T1E	T2EA	T1E	T2EA	T1E	T2EA	T1E	T2EA
PaO_2 (mmHg)	59.0 ± 9.0†	43.0 ± 11.7	57.4 ± 7.0†	40.0 ± 12.2	58.5 ± 8.9†	39.7 ± 8.6	60.1 ± 9.2†	41.4 ± 7.8	61.3 ± 6.4†	47.6 ± 6.6	59.2 ± 7.7†	42.2 ± 9.8
(kPa)	7.9 ± 1.2	5.7 ± 1.6	7.7 ± 0.9	5.3 ± 1.6	7.8 ± 1.2	5.3 ± 1.1	8.0 ± 1.2	5.5 ± 1.0	8.2 ± 0.9	6.3 ± 0.9	7.9 ± 1.0	5.6 ± 1.3
$PaCO_2$ (mmHg)	48.5 ± 6.1†	54.8 ± 3.8	48.9 ± 3.4†	57.3 ± 4.2	46.8 ± 6.5†	59.0 ± 4.3	47.2 ± 4.6†	59.4 ± 3.7	49.2 ± 5.9†	59.8 ± 4.9	48.1 ± 5.1†	58.0 ± 4.5
(kPa)	6.5 ± 0.8	7.3 ± 0.5	6.5 ± 0.5	7.6 ± 0.6	6.2 ± 0.9	7.9 ± 0.6	6.3 ± 0.6	7.9 ± 0.5	6.6 ± 0.8	8.0 ± 0.7	6.4 ± 0.7	7.7 ± 0.6
Hct (%)	33 ± 4	29 ± 3	29 ± 7	28 ± 3	29 ± 3*	27 ± 2	28 ± 4*	26 ± 3	29 ± 5*	26 ± 3	29 ± 5†	27 ± 3
Hb (mmol L^{-1})	6.9 ± 1.1	6.2 ± 0.6	6.5 ± 1.3	5.9 ± 0.6	5.9 ± 0.8*	5.7 ± 0.5	6 ± 1.2*	5.5 ± 0.5	6.5 ± 1.8†	5.5 ± 0.6	6.4 ± 1.2†	5.7 ± 0.6
pH	7.43 ± 0.03	7.40 ± 0.04	7.42 ± 0.01†	7.38 ± 0.04	7.43 ± 0.01†	7.37 ± 0.04	7.43 ± 0.01†	7.37 ± 0.03	7.43 ± 0.2†	7.37 ± 0.04	7.43 ± 0.02†	7.38 ± 0.04
BE (mmol L^{-1})	7.5 ± 1.4	8.9 ±3.7	7.2 ±1.7	8.7 ±3.3	6.1 ±3.8	8.9 ±3.5	7.3 ±2.7	9.1 ±3.1	8.0 ±2.8	9.1 ±3.4	7.2 ± 2.5†	8.9 ± 3.3
HCO_3^- (mmol L^{-1})	31.4 ± 1.9	33.2 ± 3.1	31.1 ± 1.8	33.4 ± 2.9	29.9 ± 4.0	33.7 ± 3.1	30.8 ± 3.1	33.9 ± 2.8	31.8 ± 3.1	33.9 ± 3.1	31.0 ± 2.7†	33.6 ± 2.9
Lac (mmol L^{-1})	1.5 ± 0.8	1.3 ± 0.9	1.1 ± 0.6	1.1 ± 0.6	0.9 ± 0.4	1.0 ± 0.5	0.9 ± 0.5	0.9 ± 0.4	0.8 ± 0.5*	0.8 ± 0.4	1.0 ± 0.6	1.0 ± 0.6

* Indicates intratreatment significant differences ($p < 0.05$) from values at 5 minutes.
† Indicates intertreatment significant differences at the same time point ($p < 0.05$).
‡ Indicates significant intertreatment differences between mean values ($p < 0.05$).

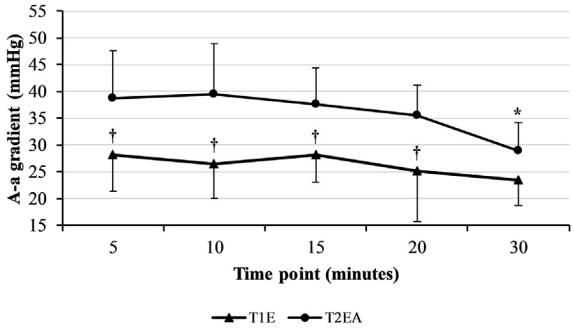

Figure 2 Mean values ± standard deviation of A-a gradients calculated from arterial blood gas samples collected from 12 adult female blesbok administered etorphine alone (0.09 mg kg^{-1}; T1E) or an etorphine–azaperone combination (0.09 mg kg^{-1} and 0.35 mg kg^{-1}; T2EA) intramuscularly. Data were collected 5 minutes postrecumbency at 5 minute intervals for a duration of 40 minutes. *Indicates intratreatment significant difference from values at 5 minutes. †Indicates significant difference between treatments at an individual time point. The *p* values were deemed significant at values ≤0.05.

gradient (Nyman et al 1990, Hedenstierna & Edmark 2010; Sarkar et al 2017). If atelectasis were to affect the majority of the lung tissue, oxygen supplementation could prove insufficient to restore normal PaO_2 levels.

In mammals, an A-a gradient greater than 10 mmHg is indicative of suboptimal alveolar-arteriolar oxygen transfer (Neary et al 2014; Sarkar et al 2017). Currently, no A-a gradient reference value is available for healthy conscious blesbok; therefore, interpreting the present study results (mean value ± SD: 24.9 ± 6.8 and 35.0 ± 8.1 mmHg following T1E and T2EA, respectively) is challenging. Etorphine administration can increase A-a gradient values owing to the development of pulmonary hypertension and alveolar-interstitial oedema, which impedes oxygen transfer to the blood by increasing the diffusion distance in domestic ruminants (Meyer et al 2015; Izwan et al 2018). We hypothesize that a similar mechanism contributed to the comparatively large A-a gradients recorded during immobilization of the blesbok in this study. However, as we did not measure pulmonary vascular pressures or determine ventilation/perfusion (V/Q) ratios, whether gas exchange impairment was due to the effect of etorphine on lung vasculature or hypoventilation-induced lung atelectasis could not be confirmed.

Anatomical differences in the lungs of some ruminant species may play an important role in limiting hypoxaemia and V/Q mismatch. The collateral ventilation system, which exists in sheep, counters V/Q mismatching by allowing normoxic air to move to hypoxic lung regions (Kuriyama & Wagner 1981; Shakespeare 2012). Conversely, cattle lack this system and are more prone to develop pulmonary hypertension and hypoxaemia (Kuriyama & Wagner 1981). Anatomy of the blesbok

lung is lacking, but if they were to resemble cattle, they could cope with hypoxia less readily than ruminant species such as sheep.

In the present study, Lac values remained within normal range (<2 mmol L^{-1}) (Pang & Boysen 2007). It is believed that drug-induced respiratory acidosis might have rapidly been counterbalanced by intracellular buffering and H^+ ion excretion by the kidneys. This would explain the increase in HCO_3^- and BE documented with both treatments and the pH values remaining within normal range (Ramadoss et al 2011).

There are no data describing the oxyhaemoglobin dissociation curve in blesbok. Our results suggest that oxygen haemoglobin dissociation curves derived from domestic ruminants may not be applicable to blesbok. In fact, the relationship between SpO_2 and PaO_2 values in blesbok seem to differ from those reported in goats. In blesbok, measured SpO_2 values correspond to lower PaO_2 values than those expected in goats (Haskins & Rezende 2006). This could be explained by a greater affinity of blesbok haemoglobin for oxygen than that of the goat. Nevertheless, further studies are needed to either confirm or refute this hypothesis.

In the present study, significant decreases in Hct and Hb were observed with the T2EA compared with T1E. This effect relates to azaperone-induced α-adrenoceptor blockade that caused vasodilation, secondary haemodilution and splenic sequestration of red blood cells (Mentaberre et al 2010).

A minor decrease in RT was observed with T2EA. This effect could be a direct result of the azaperone-related peripheral vasodilation (Swan 1993), which counteracted etorphine-induced side effects improving heat exchange (Kock & Burroughs 2012). Despite the intertreatment difference recorded and though the normal RT in blesbok is unknown, it is believed that RT remained within physiological range with both treatments in the present study.

Intertreatment differences were not documented regarding recovery times except for a statistically significant, but clinically insignificant, difference in head up time. Both treatments were associated with rapid and complete recoveries following naltrexone administration. Extrapyramidal signs were not documented during the hours following reversal, and the intertreatment washout period was deemed adequate (Walsh & Wilson 2002).

Limitations of this study include the lack of endotracheal intubation, which might have allowed ruminal fermentation gas to contaminate and increase the $P_E'CO_2$ values recorded at the level of the nasopharynx (Ding et al 2010). Therefore, in future studies immobilized animals should be intubated to avoid this confounding factor. In the present study, it was not possible to measure $\dot{V}/\dot{Q}$ ratios pulmonary vascular pressures, tidal volume or cardiac output. We believe this information would improve the understanding and interpretation of ventilation and gas exchange in the immobilized blesbok. Furthermore, the determination of oxygen delivery and consumption may provide further insight into how metabolic activity is affected by drug administration in blesbok and how this contributes to ventilatory differences between different drug treatments. In turn, further research would enable us to prevent, limit or counteract drug-induced side effects.

In conclusion, the etorphine–azaperone combination at the dose studied led to a greater level of immobilization and less systemic hypertension than etorphine alone in blesbok. Nevertheless, the drug combination had a greater detrimental impact on ventilatory function. Therefore, the authors suggest that the etorphine–azaperone combination is used judiciously and should be avoided in animals with pre-existing pulmonary disease as it may exacerbate ventilatory function. The authors recommend that supplementary oxygen administration is provided and monitoring cardiorespiratory variables be performed when immobilizing blesbok with either of these drug immobilization protocols.

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Authors' contributions

EG: study design, data management, data interpretation, statistical analysis and preparation of manuscript. LLL, JPR and GMDB: study design, data interpretation and preparation of manuscript. SP: supervising veterinarian, data interpretation and preparation of manuscript. LCH: data interpretation and preparation of manuscript.

Conflict of interest statement

Some authors of this paper are employed by the pharmaceutical company supplying the drugs and facilities used in this research.

References

Buss P, Miller M, Fuller A et al. (2016) Cardiovascular effects of etorphine, azaperone, and butorphanol combinations in chemically immobilized captive white rhinoceros (*Ceratotherium Simum*). J Zoo Wildl Med 47, 834–843.

Bowdle TA (1998) Adverse effects of opioid agonists and agonist-antagonists in anaesthesia. Drug Saf 19, 173–189.

Christie RV, Loomis AL (1932) The pressure of aqueous vapour in the alveolar air. J Physiol 3, 35–48.

Ding XZ, Long RJ, Mi JD, Guo XS (2010) Measurement of methane and carbon dioxide emissions from ruminants based on the NDIR technique. Guang Pu Xue Yu Guang Pu Fen Xi 30, 1503–1506.

Grimm KA, Lamont LA, Tranquilli WJ et al. (2015) Veterinary Anesthesia and Analgesia: The Fifth Edition of Lumb and Jones. John Wiley & Sons, Inc., Ames, IA, USA. p. 1061.

Guyenet P (2014) Regulation of breathing and autonomic outflows by chemoreceptors. Comp Physiol 4, 1511–1562.

Haigh JC (1990) Opioids in zoological medicine. J Zoo Wildl Med 21, 391–413.

Haskins S, Rezende M (2006) The caprine oxyhemoglobin dissociation curve. Res Vet Sci 80, 103–108.

Heard DJ, Kollias GV, Buss D et al. (1990) Comparative cardiovascular effects of intravenous etorphine and carfentanil in goats. J Zoo Wildl Med 21, 166–170.

Hedenstierna G, Edmark L (2010) Mechanisms of atelectasis in the perioperative period. Best Pract Res Clin Anaesthesiol 24, 157–169.

Izwan A, Snelling EP, Seymour RS et al. (2018) Ameliorating the adverse cardiorespiratory effects of chemical immobilization by inducing general anaesthesia in sheep and goats: implications for physiological studies of large wild mammals. J Comp Physiol Compar B 188, 991–1003.

Kim D, McLeod KR, Klotz JL et al. (2013) Evaluation of a rapid determination of fasting heat production and respiratory quotient in Holstein steers using the washed rumen technique1. J Anim Sci 91, 4267–4276.

Kock MD, Burroughs RE (2012) Chemical and physical restraint of wild animals. In: International Wildlife Veterinary Services (Africa) (2nd edn). Greyton, South Africa.

Kuriyama T, Wagner WW (1981) Collateral ventilation may protect against high-altitude pulmonary hypertension. J Appl Physiol 51, 1251–1256.

Lees P, Serrano S (1976) Effects of azaperone on cardiovascular and respiratory functions in the horse. Br J Pharmacol 56, 263–269.

Mentaberre G, López-Olvera JR, Casas-Díaz E et al. (2010) Use of haloperidol and azaperone for stress control in roe deer (*Capreolus capreolus*) captured by means of drive-nets. Res Vet Sci 88, 531–535.

Meyer LCR, Hetem RS, Fick LG et al. (2008) Thermal, cardiorespiratory and cortisol responses of impala (*Aepyceros melampus*) to chemical immobilisation with 4 different drug combinations. J S Afr Vet Assoc 79, 121–129.

Meyer LCR, Hetem RS, Mitchell D, Fuller A (2015) Hypoxia following etorphine administration in goats (*Capra hircus*) results more from pulmonary hypertension than from hypoventilation. BMC Vet Res 11, 18.

Neary J, Garry F, Raabis S (2014) Age-related changes in arterial blood-gas variables in Holstein calves at moderate altitude. Open Access Anim Physiol 6, 13–20.

Nyman G, Funkquist B, Kvart C et al. (1990) Atelectasis causes gas exchange impairment in the anaesthetised horse. Eq Vet J 22, 317–324.

Pang D, Boysen S (2007) Lactate in Veterinary Critical Care: Pathophysiology and Management. J Am Anim Hosp Assoc 43, 270–279.

Radcliffe RW, Ferrell ST, Childs SE (2012) Butorphanol and azaperone as a safe alternative for repeated chemical restraint in captive white rhinoceros (*Ceratotherium simum*). J Zoo Wildl Med 31, 196–200.

Ramadoss J, Stewart RH, Cudd TA (2011) Acute renal response to rapid onset respiratory acidosis. Can J Physiol Pharmacol 89, 227–231.

Riviere JE, Papich MG (2009) Veterinary Pharmacology & Therapeutics (9th edn). Wiley-Blackwell, Iowa, USA.

Roquebert J, Delgoulet C (1988) Cardiovascular effects of etorphine in rats. J Auton Pharmacol 8, 39–43.

Sarkar M, Niranjan N, Banyal P (2017) Mechanisms of hypoxemia. Lung India 34, 47–60.

Schultz HD, Li YL, Ding Y (2007) Arterial chemoreceptors and sympathetic nerve activity: implications for hypertension and heart failure. Hypertens 50, 6–13.

Semjonov A, Adrianov V, Raath JP et al. (2018) Evaluation of butorphanol–azaperone–medetomidine (BAM) in captive blesbok immobilization (*Damaliscus pygargus phillipsi*). Vet Anaesth Analg 45, 496–501.

Shakespeare A (2012) Aspiration lung disorders in bovines: A case report and review. J S Afr Vet Ass 83, 1–7.

Still J, Raath JP, Matzner L (1996) Respiratory and circulatory parameters of African elephants (*Loxodonta africana*) anaesthetised with etorphine and azaperone. J S Afr Vet Assoc 67, 123–127.

Swan GE (1993) Drugs used for the immobilization, capture, and translocation of wild animals. In: The Capture and Care Manual. McKenzie AA (ed.), The South African Veterinary Foundation. pp. 3–59. Pretoria, South Africa.

Thatcher CD, Keith JC (1986) Pregnancy-induced hypertension: Development of a model in the pregnant sheep. Am J Obstet Gynecol 155, 201–207.

Walsh VP, Wilson PR (2002) Sedation and chemical restraint of deer. N Z Vet J 50, 228–236.

Wenger S, Buss P, Joubert J et al. (2010) Evaluation of butorphanol, medetomidine and midazolam as a reversible narcotic combination in free-ranging African lions (*Panthera leo*). Vet Anaest Analg 37, 491–500.

Zeiler GE, Meyer LCR (2017) Chemical capture of impala (*Aepyceros melampus*): A review of factors contributing to morbidity and mortality. Vet Anaest Analg 44, 991–1006.

Zeiler GE, Meyer LCR (2017) Comparison of thiafentanil-medetomidine to etorphine-medetomidine immobilisation of impalas (*Aepyceros melampus*). J S Afr Vet Assoc 88, 1–8.

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