RESEARCH PAPER

Evaluation of butorphanol–azaperone–medetomidine (BAM) in captive blesbok immobilization (*Damaliscus pygargus phillipsi*)

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Abstract

Objective The fixed-dose combination of butorphanol, azaperone and medetomidine (BAM; 30, 12 and 12 mg mL^{-1}, respectively) with subsequent antagonism by naltrexone–atipamezole was evaluated for reversible immobilization of captive blesbok (*Damaliscus pygargus phillipsi*).

Study design Prospective, clinical trial.

Animals Sixteen blesbok (four males and twelve females), weighing 52.5–71.0 kg, were immobilized in South Africa.

Methods The total dose of BAM ranged from 0.5 to 0.7 mL for females and 0.7 to 0.9 mL for males. In seven animals chosen randomly, 8000 units of hyaluronidase was added to the dart. Physiologic variables were recorded every 5 minutes beginning at 10–20 minutes after darting. Arterial blood samples were collected three times at 20, 30 and 40 minutes after darting for analysis of blood acid-base status.

Results The mean administered doses of BAM were as follows: butorphanol (0.34 ± 0.08 mg kg^{-1}), azaperone (0.14 ± 0.03 mg kg^{-1}) and medetomidine (0.14 ± 0.03 mg kg^{-1}). The inductions were calm and smooth. The mean induction time was 9.6 ± 3.2 minutes with just BAM and 5.1 ± 0.8 minutes with BAM and hyaluronidase combination. Heart rate (45 ± 6 beats $minute^{-1}$) and respiratory frequency (38 ± 4 breaths $minute^{-1}$) were stable throughout immobilization. The mean arterial blood pressure for all animals was stable but elevated (137 ± 7 mmHg). Rectal temperature slightly increased over time but remained within an acceptable range. The recovery time after administering naltrexone and atipamezole was 4.8 ± 0.7 minutes.

Conclusion and clinical relevance The BAM combination proved to be reliable and effective in blesbok.

Keywords azaperone, BAM, blesbok, butorphanol, medetomidine.

Introduction

Blesbok (*Damaliscus pygargus phillipsi*) are gregarious medium-sized antelope that prefer the open grassland habitat of southern Africa. Ganhao et al. (1988) investigated the physiological responses of blesbok, eland (*Taurotragus oryx*) and red hartebeest (*Alcelaphus buselaphus*) to different capture methods, namely net capture, enclosure capture and chemical immobilization, and found that chemical immobilization elicited the lowest stress response. Chemical immobilization has become an essential part of research, in the treatment of sick or injured animals and during capture operations.

Etorphine is a widely used opioid for the chemical immobilization of blesbok (Williams & Riedesel 1987; Burroughs 1993; Kock & Burroughs 2012). Thiafentanil can also be used, and some users claim that a

mixture of etorphine and thiafentanil provides better induction than etorphine alone (Kock & Burroughs 2012). A number of sedatives and tranquilizers can also be included in the immobilizing mixture (Kock & Burroughs 2012).

One of the biggest problems with the use of powerful opioids in chemical immobilization mixtures is that they need to be highly controlled in terms of their handling, storage and record-keeping. Furthermore, these substances are not always easily accessible. Beyond these practical considerations, opioids such as thiafentanil have also been reported to be associated with hyperthermia, respiratory depression, poor muscle relaxation and capture myopathy (Mich et al. 2008).

The use of butorphanol, azaperone and medetomidine as a sedative combination provides a potentially useful alternative (Wolfe et al. 2008). Butorphanol is a synthetic opioid analgesic agent (partial agonist—antagonist), three to five times more potent than morphine. It can be combined with alpha$_2$-adrenergic agonists to produce profound sedation or light general anaesthesia (Neiffer et al. 2005). Azaperone is a short-acting neuroleptic sedative belonging to the class of butyrophenones that is often used in combination with opioids and alpha$_2$-agonists to reduce the stress from capture and handling (Kock & Burroughs 2012). Medetomidine is a potent alpha$_2$-agonist with sedative and analgesic properties that, in combination with butorphanol, provides smooth induction and good muscle relaxation. The combination of these three agents has been reported to provide safe and reversible immobilization in white-tailed deer (*Odocoileus virginianus*) (Mich et al. 2008; Miller et al. 2009; Siegal-Willott et al. 2009), rocky mountain elk (*Cervus elaphus nelsoni*) (Wolfe et al. 2014), Nubian ibex (*Capra nubiana*) (Lapid & Shilo-Benjamini 2015), black bears (*Ursus americanus*) (Wolfe et al. 2008) and African lions (Semjonov et al. 2017). Hyaluronidase is proteolytic enzyme. The effect of hyaluronidase is via enzymatic breakdown of the interstitial barrier between cells which in turn breaks down the intercellular matrix (responsible for tissue integrity) and allows drugs to reach the central compartment much faster. As a result, the rate of drug absorption is enhanced, thereby accelerating immobilization (Watson 1993; Schulenburg et al. 2007; Dittberner 2011).

The aims of this study were to evaluate the effectiveness and physiological responses of captive blesbok to BAM administered intramuscularly (IM) with and without hyaluronidase.

Material and methods

Sixteen blesbok (four males and twelve females) that required clinical examination, deworming, blood collection and genetic material collection were recruited for this study. They were housed together in enclosures on the Ngongoni private game farm at an altitude of 900 m above sea level in Mpumalanga, South Africa, and were immobilized in September 2015.

The butorphanol—azaperone—medetomideine fixed-dose combination (BAM), as used in this study, was produced by Wildlife Pharmaceuticals South Africa (Pty) Ltd. Each animal was darted with BAM. The individual dose was estimated based on animal size, and small, medium and large females were administered 0.5, 0.6 and 0.7 mL, and small, medium and large males 0.7, 0.8 and 0.9 mL, respectively. Each millilitre of the solution contained 30 mg butorphanol, 12 mg azaperone and 12 mg medetomidine. In seven randomly chosen animals of both sexes, 8000 units of hyaluronidase (Hyaluronidase Type I-S from Bovine Teste; Sigma-Aldrich, MO, USA) was added to the dart. All animals were darted between 5:00 and 12:00 or 15:00 and 17:00 hours to avoid the high, midday environmental temperatures.

A gas-powered dart gun Pneu-Dart X-Caliber (Pneu-Dart Inc., PA, USA) was used to deliver the drugs. Darts with a 2 mL capacity combined with a 19 mm long, 14 gauge needle with wire barb (Wildlife Pharmaceuticals (Pty) Ltd., South Africa) were used. Remote darting was performed in a 6 × 8 m enclosure from an upper deck of the wall at distances ranging from 5 to 12 m. All injections were administered into the femoral muscles.

To antagonize the effect of the medetomidine and butorphanol, atipamezole (Antisedan 5 mg mL^{-1}; Orion Pharma, Finland) at five times the medetomidine dose in milligrams and naltrexone hydrochloride (Trexonil 50 mg mL^{-1}; Wildlife Pharmaceuticals (Pty) Ltd., South Africa) at one time (mg to mg), the actual butorphanol dose was administered to reverse medetomidine and butorphanol, respectively. All injections were administered IM.

Monitoring and manipulations of animals

Two stages of induction were timed: stage I — from time of the darting until the first signs of sedation,

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including ears hanging down, wide stance of the thoracic and pelvic limbs and ataxia; stage II — from the injection time until sternal recumbency. Once the animals reached recumbency, an additional 2 minutes were waited before animals were approached and blindfolded. If the animal went into lateral recumbency, it was placed in sternal recumbency immediately after approaching. Animals were placed on a stretcher, carried from the enclosure and transported to a shaded area around 100 m away. The blesbok was then placed on a table in sternal position with the head fixed in a lifted position. All animals' tracheas were intubated using endotracheal tubes 10 mm in diameter. Every 5 minutes, beginning at 10–20 minutes after darting, physiological parameters were measured. A veterinary monitor, the Capnovet Deluxe Multiparameter Monitor (Eickemeyer, Germany), was used to register heart rate (HR) and respiratory frequency (f_R), oxygen saturation (SpO_2), end-tidal carbon dioxide ($P_E'CO_2$), noninvasive arterial blood pressure [systolic (SBP), diastolic (DBP) and mean (MBP)] and body temperature. The pulse oximeter transducer was fixed on the tongue of the animal. The capnograph transducer was attached to the endotracheal tube. The temperature transducer was inserted into the rectum. The noninvasive blood pressure (NIBP) measuring cuff (Criticon Soft-Cuf nr 5, 8 × 15 cm; GE Healthcare, NY, USA) was placed on the thoracic limb. Auscultation with a stethoscope (3M Littmann Classic II S.E. Stethoscope; 3M, MN, USA) was performed every 5 minutes for the entire duration of anaesthesia. The level of muscle relaxation was subjectively assessed based on the general muscle tone on a 3-point scale: level 1 — the absence of muscle tone; level 2 — a light tone; and level 3 — a marked tone. Capillary refill time was evaluated on the mucus membranes of the maxilla. Either the presence or absence of the palpebral reflex was additionally registered. Animals were observed for rumen tympani throughout the study period.

Three arterial blood samples were collected from each blesbok at 20, 30 and 40 minutes after darting using the auricular artery. The puncture was performed anaerobically using a heparinized syringe and a 21 gauge needle. Blood sample analysis was conducted immediately using a portable analyzer (i-STAT 1 Portable Clinical Analyzer; Abaxis, CA, USA) and cartridges (i-STAT cartridges CG4+, CHEM8+; Abaxis). Variables measured from arterial blood included pH, partial pressure of arterial oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), lactate, haematocrit, sodium, potassium, chloride, urea, creatinine, glucose and ionized calcium levels. Actual base excess, actual bicarbonate, oxygen saturation and haemoglobin were calculated automatically from the measured values by the portable analyzer.

The duration of immobilization was 50 minutes. The animals were extubated 40 minutes after the beginning of anaesthesia. After extubation, all animals were weighed using a portable scale (Anyload OCSL Mini Crane Scale; Anyload Transducer Co. Ltd., BC, Canada) to measure their body mass, and transported back to the enclosure. In the enclosure, the blesbok were placed in sternal position on the ground. Antagonists were then injected IM into the femoral muscle region. The following stages of recovery were recorded: time elapsed from injection until the first signs of recovery, including eye blinking, time to head lifting and time to standing.

Statistical analysis

For the analysis of anaesthetic dosage effects and the effect of hyaluronidase addition on the blesboks' HR, f_R, SBP, DBP, MBP, SpO_2, $P_E'CO_2$, PaO_2 and $PaCO_2$, the area under the curve (AUC) was calculated using a trapezoid method for every measurement during the immobilization period (50 minutes). The mean AUCs were used as response variables in linear regression models. The exact anaesthetic dosage (calculated after weighting of immobilized blesbok) and body weight were used as continuous explanatory variables. Hyaluronidase (yes, $n = 7$; no, $n = 9$) and sex (male, $n = 4$; female, $n = 12$) were added into every model as two-level categorical variables.

For the analysis of the effect of hyaluronidase and BAM on induction (time to recumbency) and recovery, time linear regression models were used. Inverse transformation from induction time (to achieve normal distribution of model residuals) was used.

Linear mixed models were used to explore the overall time trend in lactate, arterial blood pH and body temperature and differences in time trend between the hyaluronidase administration groups. Blesbok were included as random intercepts and polynomials of time (minutes), with interactions with the hyaluronidase group added as fixed effects in increasing order. The overall time trend differences between groups were tested with an F test. Isotropic spatial exponential covariance structure was used to model serial correlations of repeated measurements at the within-animal level in all models.

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A backward elimination procedure was performed for the all final models, and biologically meaningful interactions were tested. The model's assumptions were verified by scatter and normality plots of standardized residuals. For statistical analysis, STATA 14.0 software (Stata Corporation, TX, USA) was used. A p value ≤ 0.05 was considered statistically significant. Data are presented as median (range) and mean ± standard deviation.

Results

Data from four male animals weighing 66.5 (52.5−69.3) kg and from 12 female animals weighing 60.8 (52.0−71.0) kg were used in this study. All 16 blesbok injected with BAM were successfully immobilized. There was no need for additional injections to achieve immobilization. The following dose rates were used: 0.017 ± 0.003 mL kg^{-1} or 0.17 ± 0.03 mL 10 kg^{-1}. Total dose ranged from 0.5 to 0.9 mL. The actual doses were as follows: butorphanol (0.34 ± 0.08 mg kg^{-1}), azaperone (0.14 ± 0.03 mg kg^{-1}) and medetomidine (0.14 ± 0.03 mg kg^{-1}). The inductions were calm and smooth. All animals showed first signs of sedation, including drooping ears, eyelids and heads, within 3.4 ± 0.9 minutes after drug administration. The addition of hyaluronidase to the dart decreased the time to first sign of sedation from 3.8 ± 0.9 minutes (animals immobilized with only BAM) to 2.9 ± 0.6 minutes (animals immobilized with BAM and hyaluronidase) (p = 0.001). There was also a difference in induction time between animals immobilized with pure BAM (9.6 ± 3.2 minutes) and animals immobilized with BAM and hyaluronidase (5.1 ± 0.8 minutes) (p = 0.001) (Table 1). There was no correlation between the variations in the range of induction times recorded and the BAM dose (p = 0.285) or between the induction times and the body weight of animals (p = 0.917).

The quality of immobilization was considered good based on muscle relaxation, the absence of muscle twitching and the lack of significant response to intubation, painful stimuli (drawing blood) and handling. Capillary refill time in all animals did not exceed 2 seconds.

Table 2 presents the main monitoring variables including HR, f_R, SpO_2, $P_E'CO_2$, arterial acid-base balance and ventilation parameters and rectal body temperature during chemical restraint. There were no differences between the BAM and BAM with hyaluronidase groups. All parameters were considered acceptable for this species.

The rectal body temperatures of all animals increased slightly during immobilization but remained within an acceptable range (37−40 °C). No animals demonstrated ruminal distention or excessive salivation during immobilization.

All animals showed increased respiratory rates and mild hypoxaemia (PaO_2 <80 mmHg) (Fahlman 2014). Lactate levels steadily declined in all animals during immobilization time ($p < 0.001$). In animals immobilized with BAM and hyaluronidase, the lactate declined more steadily (interaction term $p = 0.01$).

All animals typically recovered within less than 5 minutes of administration of naltrexone and atipamezole. There was no difference in recovery time between animals receiving only BAM and animals receiving BAM with hyaluronidase (p = 0.811) (Table 1). Some signs of sedation were still observed in all animals within the first 5 minutes after standing.

Discussion

The present study indicates that BAM (butorphanol−azaperone−medetomidine) is an efficient immobilization drug for blesbok. The addition of hyaluronidase to the dart mixture accelerated induction

Table 1 Mean ± standard deviation (SD) of induction and recovery times of blesbok, immobilized with BAM (butorphanol−azaperone−medetomidine) and BAM combined with hyaluronidase

	Group	
	BAM (n = 9)	**BAM and hyaluronidase (n = 7)**
Time to first sign of sedation (minutes)	3.8 ± 0.9	2.9 ± 0.7
Time to recumbency (minutes)	9.6 ± 3.2	5.1 ± 0.8
Time from injection of antidotes to first sign of recovery (minutes)	3.6 ± 0.9	3.5 ± 0.6
Time to standing (minutes)	4.8 ± 0.7	4.9 ± 0.5

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Table 2 Physiologic variables and arterial blood gases in captive blesbok darted with BAM (butorphanol-azaperone-medetomidine). Mean values are presented for the periods of time 10–20, 20–30 and 30–40 minutes after darting and for the entire immobilization (overall). Results are presented as mean ± standard deviation and (range)

Variable		Timepoint (minutes)			
		10–20	20–30	30–40	Overall
HR	beats minute^{-1}	45 ± 7	44 ± 8	45 ± 7	45 ± 6 (36–55)
f_R	breaths minute^{-1}	37 ± 5	37 ± 5	39 ± 5	38 ± 4 (30–46)
T	°C	38.9 ± 0.8	39.1 ± 0.9	39.1 ± 1	39.1 ± 0.9 (37.1–40.0)
SAP	mmHg	169 ± 14	167 ± 12	161 ± 16	166 ± 11 (150–190)
DAP	mmHg	118 ± 7	118 ± 6	116 ± 7	118 ± 3 (113–123)
MAP	mmHg	140 ± 9	136 ± 10	135 ± 10	137 ± 7 (127–151)
SpO_2	%	90 ± 3	92 ± 2	93 ± 3	93 ± 2 (89–96)
PaO_2	mmHg	72 ± 3	76 ± 2	73 ± 3	72 ± 3 (68–78)
	kPa	9.6 ± 0.4	10.1 ± 0.3	9.7 ± 0.4	9.6 ± 0.4 (9.1–10.4)
$P_{E'}CO_2$	mmHg	41 ± 4	41 ± 3	41 ± 3	41 ± 4 (32–49)
$PaCO_2$	mmHg	45.0 ± 2.5	45.5 ± 1.9	46 ± 2.9	45 ± 2.5 (41–49)
	kPa	6.0 ± 0.3	6.1 ± 0.3	6.1 ± 0.4	6.0 ± 0.3 (5.5–6.5)
pH		7.42 ± 0.04	7.44 ± 0.03	7.45 ± 0.04	7.44 ± 0.04 (7.36–7.5)
Lactate	mmol L^{-1}	1.59 ± 0.96	1.32 ± 0.83	1.13 ± 0.73	1.32 ± 0.83 (0.44–3.06)

HR, heart rate; f_R, respiratory rate; T, Rectal temperature; SAP, systolic blood pressure; DAP, diastolic blood pressure; MAP, men blood pressure; SpO_2, haemoglobin oxygen saturation; PaO_2, partial pressure of oxygen; $P_{E'}CO_2$, end-tidal carbon dioxide; $PaCO_2$, partial pressure of carbon dioxide.

times, both in time to first sign of sedation and time to recumbency. This was expected since the addition of hyaluronidase has been shown to increase drug absorption and induction times (Bush et al. 2004; Cattet & Obbard 2010; Dittberner 2011; Dittberner et al. 2015).

All blesbok showed elevated respiratory rates and mild hypoxaemia throughout immobilization. This may be a direct effect of butorphanol. Mich et al. (2008) reported hypoxaemia in white-tailed deer (*Odocoileus virginianus*) immobilized with butorphanol, azaperone and medetomidine. The authors attributed this to increased venous admixture as a result of a lower ventilation/perfusion (V/Q) ratio and increased physiologic shunting because of both opioid and alpha$_2$-agonist administration. Wolfe et al. (2014) reported similar results in Rocky Mountain elk immobilized with butorphanol, azaperone and medetomidine and noted that hypoxaemia was most severe in animals that received high doses of the combination.

In all cases, animals were bradycardic (HR <55 beats minute^{-1}) as well as hypertensive. This is possibly resulting from medetomidine increasing initially the blood pressure as a result of peripheral vasoconstriction. The observed reflex bradycardia is therefore likely secondary to the medetomidine-induced hypertension. Although azaperone is reported to have a hypotensive effect because of a reduction in total peripheral resistance, no such effect was observed in these blesbok (Clarke 1969; Lees & Serrano 1976; Serrano & Lees 1976; Hattingh et al. 1994). This effect may be species-specific or perhaps was superseded by the hypertensive effect of medetomidine.

The main limitation of the present study was the small sample size of animals which decreases the statistical power of the study. All animals used in the study were in good condition and clinically healthy, and information about the effect of BAM on starving or injured animals is not available.

In conclusion, the BAM combination at the doses used in this study proved to be a reliable immobilization agent and an alternative for ultra-potent opioids for captive blesbok. Advantages of BAM include a small drug volume for darting, calm and smooth induction, long duration of immobilization and the ability to reverse the effects of immobilization with naltrexone and atipamezole. The addition of hyaluronidase to the dart mixture is advisable to decrease the induction times in cases of chasing wild animals. Physiological parameters should be monitored throughout chemical restraint, and oxygen supplementation may be necessary with this drug combination.

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Authors' contributions

All authors participated in the conception and design of the study, or acquisition of data, or interpretation of data as well as in drafting of the article or revising it critically. All authors approved the final version of the manuscript.

Conflict of interest statement

Dr JP Raath is the owner of Wildlife Pharmaceuticals South Africa (Pty).

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