SHORT COMMUNICATION

Evaluation of butorphanol-azaperone-medetomidine in captive cheetah (*Acinonyx jubatus*) immobilization

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Abstract

Objective The butorphanol-azaperone-medetomidine fixed-dose combination (BAM, respectively, 30-12-12 mg mL^{-1}) with subsequent antagonism by naltrexone-atipamezole was evaluated for reversible immobilization of captive cheetahs (*Acinonyx jubatus*).

Study design Prospective, clinical trial.

Animals Twelve cheetahs (six males and six females, weighing 37–57 kg) housed in enclosures, were immobilized at Hoedspruit Endangered Species Centre in the Republic of South Africa.

Methods BAM volume dose rate was 0.009–0.014 mL kg^{-1} (mean ± standard deviation 0.010 ± 0.001 mL kg^{-1}). Total dose in all animals was 0.5 mL. The actual doses were as follows: butorphanol (0.29 ± 0.04 mg kg^{-1}), azaperone (0.12 ± 0.01 mg kg^{-1}) and medetomidine (0.12 ± 0.01 mg kg^{-1}). Physiologic variables and quality of immobilization were recorded every 5 minutes beginning at 15–20 minutes after darting. Arterial blood samples were collected three times at 20, 30 and 40 minutes after darting from all animals for analysis of blood oxygenation and acid-base status.

Results The inductions were calm and smooth and mean induction time was 4.0 ± 1.1 minutes. Heart rate (50 ± 9 beats $minute^{-1}$) and respiratory frequency (20 ± 3 breaths $minute^{-1}$) were stable throughout immobilization. The recovery time after reversing with naltrexone and atipamezole was 9.1 ± 3.6 minutes.

Conclusions and clinical relevance BAM proved to be a reliable and cardiovascular stable drug combination for immobilization of cheetahs.

Keywords Azaperone, BAM, butorphanol, cheetah, immobilisation, medetomidine.

Introduction

Free ranging cheetah (*Acinonyx jubatus*) populations are listed as vulnerable on the International Union for Conservation of Nature red list with population numbers continuously declining in Africa (Durant et al. 2015). Chemical immobilization of cheetahs is often performed in relocation and breeding projects where animals require immobilization for routine management practices such as vaccinations and injury or disease treatment.

Although published reports on the use of immobilizing drugs in cheetahs is limited, some published studies have investigated a number of drugs and/or drug combinations. Recently, a number of combination drugs have been reported for immobilization in this species. Janssens et al. (1994) used a combination of ketamine and xylazine in a female cheetah.

Lewandowski et al. (2002) evaluated the use of tiletamine-zolazepam (TZ) in combination with ketamine and xylazine at various dosages. The combination provided rapid induction after a single intramuscular (IM) injection along with a safe, predictable working time, good muscle relaxation and analgesia. Stegmann & Jago (2006) compared the effects of either ketamine and medetomidine (KM), ketamine and midazolam (K/MID) or tiletamine, zolazepam and medetomidine (TZM). Although no differences were observed in induction time or blood pressure (BP) between the groups, the authors noted seizures in all animals immobilized with KM as well as a lower heart rate (HR).

The use of the TZ has been the popular choice for many veterinarians working with cheetahs in South Africa (Lewandowski et al. 2002; Walzer & Huber 2002; Stegmann & Jago 2006). However, without an antagonist, recovery usually takes a long time and while some authors report a smooth recovery, others have found that recovery is often rough with animals jerking their heads around and repeatedly hitting the floor for up to 30 minutes (Walzer & Huber 2002). Walzer & Huber (2002) found that the use of a partial antagonist such as flumazenil or sarmazenil shortened the recovery time and lead to a calmer and smoother recovery.

Most notably, reports on the majority of immobilization protocols mentioned that cheetahs could not be rapidly reversed, therefore animals were vulnerable to attack should they be released into the wild during recovery.

Lafortune et al. (2005) investigated the use of medetomidine, butorphanol and midazolam in captive cheetahs and found this combination to produce a smooth and fast induction, adequate immobilization for minor procedures and a quick recovery after reversal with atipamezole, flumazenil and naltrexone. The authors noted that this ketamine-free combination was particularly beneficial for animals with liver/kidney dysfunction. The aim of the current study was to investigate the use of a butorphanol-azaperone-medetomidine fixed-dose combination (BAM) for the safe and reversible immobilization of cheetahs. The study hypothesized that this combination could produce both rapid and smooth inductions as well as induce a good level of immobilization characterized by stable cardiopulmonary and respiratory parameters. In addition, the study aimed to show that reversal of this combination with naltrexone and atipamezole could produce rapid, uneventful recoveries that would be suitable for use in wild animals.

Materials and methods

Twelve cheetahs (six males and six females), housed in enclosures, were immobilized at Hoedspruit Endangered Species Centre in the Republic of South Africa during January 2016. The animals were immobilized for clinical examination, blood collection, microchipping, deworming and genetic material collection.

BAM, as used in this study, was produced by Wildlife Pharmaceuticals South Africa (Pty) Ltd., South Africa. Each mL of the solution contained the active pharmaceutical ingredients as 30 mg of butorphanol, 12 mg of azaperone and 12 mg of medetomidine. The individual doses for the combination were calculated based on a previous BAM study on African lions (Semjonov et al. 2017). The total dose in all animals was 0.5 mL. The body weight of the animals was estimated based on body condition. A pistol projector (Dan-Inject ApS, Denmark) was used to deliver the drug. Darts (Dan-Inject ApS) with a volume of 1.5 mL and a 1.5 × 30 mm plain needle were used (Dan-Inject ApS). Remote darting was performed from outside the enclosures at distances ranging from 3 to 5 m. All injections were administered into the muscles of the thigh. All the animals were darted between 6 am and 10 am, avoiding high mid-day environmental temperatures. The air temperature ranged from 22.0 to 38.6 °C.

Two stages of induction were timed: first from time of the darting until the first signs of sedation, including open mouth, ataxic gait and lowering of the head; second from the injection time until sternal or lateral recumbency. Once the animals reached lateral recumbency, an additional 5 minutes were given before animals were approached and blindfolded. Animals were placed on a stretcher, carried from the enclosure and transported by vehicle to a shaded area around 300 m from the enclosure, where monitoring could be performed. The tracheas of all animals were intubated using endotracheal tubes (Jorgensen Labs, CO, USA) 9−11 mm in diameter. Every 5 minutes, beginning at 15−20 minutes after darting, monitoring of physiological parameters [HR, respiratory frequency (f_R), oxygen saturation (SpO_2), noninvasive BP and rectal temperature] was conducted using a veterinary monitor (Capnovet Deluxe Multiparameter Monitor; Eickemeyer, Germany). Auscultation with a

stethoscope (3M Littmann Classic II S.E. Stethoscope; 3M United States, MN, USA) was performed every 5 minutes for the entire period of immobilization. The level of muscle relaxation was assessed based on the general muscle tone and position of the lower jaw using a 3-point scale. Level 1 indicated the absence of muscle tone, level 2—a light tone and level 3—a strongly marked tone. Capillary refill time and palpebral reflex were additionally monitored.

Arterial blood samples were collected from the femoral or median caudal artery of the tail at 20, 30 and 40 minutes after darting. The samples were immediately analysed using a portable analyzer (i-STAT1 Portable Clinical Analyzer; Abaxis, CA, USA) and cartridges (i-STAT cartridges CG4+ & CHEM8+; Abaxis). Variables measured included pH, arterial partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), lactate, haematocrit, sodium, potassium, chlorine, urea, creatinine, glucose and ionized calcium levels. Actual base excess, actual bicarbonate, arterial haemoglobin, arterial blood SpO_2 and haemoglobin were calculated from the measured values.

The average duration of monitoring and of all the manipulations was 40 minutes. The animals were extubated 45−50 minutes after the beginning of immobilization, provided a strongly marked palpebral reflex was observed. Following extubation, all animals were weighed using a portable scale (Anyload OCSL Mini Crane Scale; Anyload Transducer Co. Ltd., Canada) to determine their body weight and transported back to the enclosure by vehicle. In the enclosure the cheetahs were placed in the lateral position in the shade on the ground.

To reverse the effect of medetomidine, atipamezole (Antisedan 5 mg mL^{-1}; Orion Pharma, Finland) at five times the medetomidine dose in mg was used. Naltrexone hydrochloride [Trexonil 50 mg mL^{-1}, Wildlife Pharmaceuticals (Pty) Ltd.] was used to reverse butorphanol at a total dose of 15 mg in all animals. Antagonists were then injected IM into the muscles of the thigh. The following stages of recovery were recorded: time elapsed from injection until the first signs of recovery, including eye blinking; time to head lifting; time to standing; and time to fully coordinated movement (i.e. full recovery).

Statistical analysis

For the analysis of anaesthetic dosage effects on the cheetahs' HR, f_R, systolic and diastolic BP, mean arterial pressure (MAP), SpO_2, PaO_2 and $PaCO_2$, the area under the curve (AUC) was calculated using a trapezoid method for every measurement for the immobilization period (40 minutes). The mean AUCs were used as response variables in linear regression models. The exact anaesthetic dosage (calculated after weighing immobilized cheetahs) and bodyweight were used as continuous explanatory variables. Sex was included in models to control for a possible cofounding effect. For evaluation f_R effect on the PaO_2 and SpO_2 linear egression models using PaO_2 or SpO_2, mean AUCs as response variables and f_R as an explanatory variable were used.

For analysis of the effect of BAM on induction (time to recumbency) and recovery time, linear regression models were used.

Linear mixed models were used to explore the overall time trend in lactate, arterial blood pH and body temperature. Animals were included as random intercepts and polynomials of time (minutes) as fixed effects in increasing order. The isotropic spatial exponential covariance structure was used to model serial correlations of repeated measurements at the within-animal level in all models.

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

Results

The average duration of monitoring and of all manipulations was 40 minutes. Data from six male cheetahs weighing 54.1 ± 2.8 kg (range 50−57 kg) and from six female animals weighing 47.8 ± 5.6 kg (range 37−54 kg) were used in this study. In all 12 animals, immobilization occurred after a single injection of BAM. There was no need for additional injections to achieve immobilization. The following actual dose rates were used: BAM volume dose rate range was 0.009−0.014 mL kg^{-1} (0.010 ± 0.001 mL kg^{-1}). The total dose in all animals was 0.5 mL. The actual doses were as follows: butorphanol (0.29 ± 0.04 mg kg^{-1}), azaperone (0.12 ± 0.01 mg kg^{-1}) and medetomidine (0.12 ± 0.01 mg kg^{-1}). First signs were observed within 1−5 minutes (2 ± 1 minutes) after darting and all animals were recumbent between 3 and 7 minutes (4 ± 1 minutes) after darting.

There was no association between the BAM actual dose and the induction time ($p = 0.751$) recorded. The inductions were observed to be calm and smooth with no side effects. Vomiting was not observed in any of the cheetahs. Two animals remained in sternal recumbency and never attained lateral recumbency but were immobilized; 10 cheetahs went into lateral recumbency.

Immobilization was stable and no sudden arousals were observed. During the first 20–30 minutes of immobilization, spontaneous limb twitches were present in nine animals. Low jaw muscle tone disappeared within 10–15 minutes after injection and remained at level 1. Capillary refill times were less than 2 seconds in all animals. In seven animals, the colour of the tongue was bluish, while breathing was deep and stable at the same time. Palpebral reflex disappeared at the fifteenth minute of monitoring and did not reappear until recovery. None of the cheetahs showed any reaction to intubation, extubation, or other painful procedures (e.g. blood collection). No apnoea was observed in any of the cheetahs, but abnormal breathing patterns, characterized by inhalation followed by a pause in breathing and eventual exhalation, were recorded in five animals. The arterial blood gases were at an acceptable level throughout immobilization [PaO_2 69 ± 9 mmHg (9.2 ± 1.2 kPa) and $PaCO_2$ 33 ± 5 mmHg (4.4 ± 0.7 kPa)]. There was no association between f_R and PaO_2 ($p = 0.874$) or f_R and SpO_2 ($p = 0.0768$). The duration of immobilization of each cheetah without additional doses was little less than 1 hour. Recovery was smooth and calm. Time from injection of the antidotes to the first signs of recovery was 4.5 ± 1.7 minutes; time of full recovery was 9.0 ± 3.6 minutes. There was no correlation between recovery time and the actual BAM dose ($p = 0.854$). No mortalities occurred during the study.

Table 1 presents the mean ± SD and range of the main parameters measured during monitoring and the results of blood gases analyses. Blood pH levels ($p = 0.023$) and lactate levels ($p < 0.001$) steadily declined in all animals, which is indicative of normal tissue perfusion. MAP was elevated (167 ± 19 mmHg) and there was no association between MAP and the actual BAM dose ($p = 0.640$).

Discussion

Published reports on suitable immobilization protocols for cheetahs are very limited, although immobilization of this species is common, particularly in South Africa. Additionally, most studies report on the use of TZ or ketamine combinations. When animals are to be released into the wild, a fully reversible

Table 1 The physiological response of cheetahs to immobilization with butorphanol-azaperone-medetomidine fixed-dose combination (BAM) over a 40-minute period.

Variable		10–20 min[1]	20–30 min[1]	30–40 min[1]	Overall*[min-max]
Heart rate	Beats minute^{-1}	48 ± 10	50 ± 10	53 ± 9	50 ± 9 [32–70]
Respiratory frequency	Beats minute^{-1}	18 ± 3	19 ± 4	18 ± 3	20 ± 3 [8–28]
Rectal temperature	°C	38.1 ± 0.6	38.2 ± 0.7	38.3 ± 0.7	38.2 ± 0.7 [36.8–39.1]
Systolic blood pressure	mmHg	198 ± 22	197 ± 20	188 ± 18	197 ± 19 [122–209]
Diastolic blood pressure	mmHg	152 ± 20	151 ± 21	147 ± 16	151 ± 19 [96–176]
Mean arterial pressure	mmHg	172 ± 20	168 ± 21	163 ± 16	167 ± 19 [106–186]
SpO_2	%	88 ± 5	90 ± 4	91 ± 4	93 ± 2 [80–100]
SO_2	%	93 ± 4	90 ± 5	91 ± 4	92 ± 4 [79–95]
PaO_2	mmHg (kPa)	72 ± 3 (9.6 ± 0.4)	76 ± 2 (10.1 ± 0.3)	73 ± 3 (9.7 ± 0.4)	68 ± 9 [50–86] (9.1 ± 1.2 [6.7–11.5])
$PaCO_2$	mmHg (kPa)	33 ± 5 (4.4 ± 0.7)	34 ± 5 (4.5 ± 0.7)	32 ± 5 (4.3 ± 0.7)	33 ± 5 [29–45] (4.4 ± 0.7 [3.9–6.0])
pH[†]		7.35 ± 0.03	7.33 ± 0.02	7.32 ± 0.05	7.33 ± 0.02 [7.25–7.41]
Lactate	mmol L^{-1}	0.42 ± 0.4	0.30 ± 0.2	0.30 ± 0.2	0.3 ± 0.2 [0.30–0.88]

*Median ± standard deviation. [†]Corrected to the rectal temperature.

PaO_2, arterial partial pressure of oxygen (measured value, temperature corrected); $PaCO_2$, partial pressure of arterial carbon dioxide (measured value, temperature corrected); SpO_2, haemoglobin oxygen saturation measured by pulse oximetry.

drug combination which provides rapid, smooth recoveries is important, so as not to endanger the welfare of the animals when they are released into areas where they are vulnerable to attack by other predators. Moreover, rapid recoveries allow for more complete monitoring of recovery upon release of the animal.

In the current study, all the animals showed calm and smooth inductions that were rapid and without observable side effects. The lack of intramuscular and subcutaneous fat as well as the excellent muscular blood supply in this species likely contributed to the rapid inductions. Induction times were similar to those reported for cheetahs immobilized with TZ (Deem et al. 1998; Lewandowski et al. 2002; Walzer & Huber 2002) and much faster than those that have been reported for cheetahs immobilized with KM (9.2 ± 3.4 minutes), ketamine, midazolam and medetomidine (11.3 ± 10 minutes) or TZM (16.8 ± 18.1 minutes) (Stegmann & Jago 2006).

Seven animals presented with bluish coloured tongues or mild cyanosis. Cyanosis is caused by the presence of more than 5 g desaturated haemoglobin per 100 mL of blood and is generally accepted to develop when blood is insufficiently oxygenated in the lungs or when haemoglobin is unable to carry oxygen (Sinclair 2003). It has been reported in a number of species treated with medetomidine, and Miller et al. (2009) note that oral mucous membranes should consistently be monitored as an adjunct to pulse oximetry, specifically when low SpO_2 values are observed during immobilization with BAM. This is because of the peripheral vasoconstrictive effects of the alpha2-adrenoceptor, medetomidine, in the drug combination (Flacke 1992). Additionally, Sinclair (2003) theorizes that cyanosis may also develop because of the stagnation of blood within peripheral capillary beds, which results in increased oxygen extraction. Thus, the cyanosis observed with the administration of medetomidine may likely be due to low blood flow through peripheral capillary beds and an actual venous desaturation. This is substantiated by the fact that relatively low peripheral SpO_2 was observed in all the animals ($SpO_2 < 95\%$) while arterial blood gas analysis revealed that blood oxygenation was at an acceptable level throughout immobilization ($PaO_2 > 70$ mmHg). It must be kept in mind that pulse oximetry readings may often be problematic, particularly in animals treated with alpha$_2$-agonists that result in vasoconstriction, since the decreased peripheral blood flow may hamper accurate readings.

Respiration was good with all animals maintaining respiratory rates between 10 and 20 breaths minute^{-1}, consistent PaO_2 values > 70 and $PaCO_2$ values < 40 mmHg throughout immobilization. Although none of the animals developed apnoea, some apneustic-type breathing was noted in five of the animals. This abnormal breathing pattern was characterized by inhalation followed by a pause in breathing and eventual exhalation, although neither inhalation nor exhalation appeared abnormal in depth or duration. This occurrence may have been due to a respiratory disturbance as a result of both butorphanol and medetomidine, although no published reports are available that elucidate on the effect of butorphanol in combination with medetomidine on breathing patterns. Doses of 20–60 μg kg^{-1} of medetomidine have been reported to result in reduced respiratory rates for varying periods in a number of studies in dogs (Sinclair 2003). Conversely, butorphanol is also a sigma-receptor agonist which may stimulate respiratory drive. In the current study, respiratory rates and blood gas results indicated that respiration was adequately maintained throughout monitoring and so clinically, the occurrence of apneustic-type breathing in some of the animals could be considered irrelevant.

Hypertension was noted in all animals with MAP consistently exceeding 150 mmHg. Similar results were reported by Deem et al. (1998) in cheetahs immobilized with TZM. Lafortune et al. (2005) also noted hypertension in all the cheetahs they immobilized with a medetomidine-butorphanol-midazolam combination. The peripheral vasoconstriction caused by medetomidine has been shown to result in hypertension, although this effect has also been reported to be transient (Sinclair 2003). Additionally, this effect may vary between species and although the extent of the effect is not dose-dependent, its duration has been shown to increase with increasing doses of medetomidine (Kuusela et al. 2000) . In dogs, Kuusela et al. (2000) found that medetomidine doses exceeding 20 μg kg^{-1} resulted in a longer duration of hypertension associated with a persistent increase in systemic vascular resistance. In the current study, the mean dose of medetomidine in the cheetah was 120 μg kg^{-1} which could explain why hypertension was noted throughout monitoring. Furthermore, it has been suggested that cheetahs in captivity suffer from chronic stress which may, as it does in humans, produce hypertension (Cassia et al. 2015). Indeed, Stegman and Jago (2006) found that immobilized

captive cheetahs were hypertensive whether KM was used or whether K/MID was used, suggesting that medetomidine was not the only contributor to the high BP observed.

Overall, BAM at a dose of 0.010 ± 0.001 mL kg^{-1} or 0.5 mL per adult cheetah produced a safe and reliable immobilization in cheetahs with no re-dosing required to maintain immobilization for up to 50 minutes. Inductions and recoveries were smooth and uneventful, and no sudden arousals were observed in any of the animals during immobilization. Cardiovascular and respiratory parameters fell within acceptable ranges. Although hypertension was noted in most of the animals, this has been reported by a number of authors in cheetahs immobilized with different drug protocols.

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Conflict of interest statement

Authors declare no conflict of interest. Dr JP Raath is the owner of Wildlife Pharmaceuticals South Africa (Pty).

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