EVALUATING THE USE OF A BUTORPHANOL-AZAPERONE-MEDETOMIDINE FIXED-DOSE COMBINATION FOR STANDING SEDATION IN AFRICAN ELEPHANTS (LOXODONTA AFRICANA)

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EVALUATING THE USE OF A BUTORPHANOL-AZAPERONE-MEDETOMIDINE FIXED-DOSE COMBINATION FOR STANDING SEDATION IN AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*)

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Abstract: This study investigated the use of a fixed-dose combination of 30 mg/ml butorphanol, 12 mg/ml azaperone, and 12 mg/ml medetomidine for the standing sedation of captive African elephants (*Loxodonta africana*). In total, seven females (mean age 19.6 yr; range 6–31 yr) and six males (mean age 33.5 yr; range 9–35 yr) were sedated. The estimated dose was 0.0005 ± 0.0001 ml/kg and 0.006 ± 0.001 ml/cm shoulder height, which resulted in a dose of 0.016 ± 0.002 mg/kg or 0.19 ± 0.04 mg/cm shoulder height butorphanol, 0.006 ± 0.0008 mg/kg or 0.076 ± 0.015 mg/cm shoulder height azaperone, and 0.006 ± 0.0008 mg/kg or 0.076 ± 0.015 mg/cm medetomidine. First signs of sedation were observed within 3–10 min (mean 6 ± 2 min) after darting, and monitoring of the animals started on average at 24 ± 9 min after darting. No bradycardia was observed in any of the elephants (mean heart rate 40.0 ± 6.55 beats/min), although all the animals were mildly hypotensive (mean blood pressure 118.5/86 [94.5]). Rectal temperatures fell within acceptable ranges, and respiratory parameters were stable in all the animals throughout sedation and fell within the standard ranges reported for conscious, standing elephants. Only one elephant had clinically significant hypoxemia characterized by a partial pressure of oxygen (PaO_2) < 60 mm Hg. This elephant was also hypercapnic ($PaCO_2$ > 50 mm Hg), although pH and peripheral capillary oxygen saturation fell within acceptable ranges. None of the elephants reacted to moderately painful stimuli while sedated. The combination was reversed with intramuscular injections of naltrexone (1 mg for every 1 mg butorphanol) and atipamezole (5 mg for every 1 mg medetomidine). Recovery was smooth and calm in all the animals. Time from injection of the reversals until the first signs of recovery was 4.6 ± 2.01 min (range 1–8 min).

INTRODUCTION

Captive African elephants (*Loxodonta africana*), such as those in zoos, sanctuaries, and rehabilitation centers, regularly require veterinary interventions. Sedation, chemical restraint, or both is therefore necessary when elephants are not trained to cooperate for a procedure, when adequate handling facilities are lacking, or when painful procedures have to be carried out. Sedation not only makes the experience less traumatic for the elephants, but it also allows for safer access and handling of captive elephants.[23,30,32] In the wild, African elephants are usually anesthetized with a potent opioid, such as etorphine or thiafentanil, which results in complete recumbency of the animals to handle them safely.[14,45,46,53] However, in captive elephants, many procedures can be performed without the need for lateral recumbency. Standing sedation results in less stress in group-housed animals because the remaining animals in the herd are not witness to an unnaturally recumbent conspecific and are often unaware that the sedated animal underwent a medical procedure.[30] Standing sedation also allows for access to both sides of the animal and eliminates the potential risks involved with inappropriate recumbent positioning. When elephants are immobilized with potent opioids to achieve complete lateral recumbency, not only can recumbency with the head down on a slope lead to compression of the lungs by the temporary displacement of internal organs, but obstruction of the upper airways by foreign objects or the positioning of the trunk under the body can be fatal. Nerve compression and muscle damage by a hard surface or by rocks are an additional physical risk to elephants going into lateral recumbency. Additionally, potent opioids that have to be used to achieve reliable lateral recumbency in ele-

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phants have also been reported to lead to pulmonary hypertension, resulting in potentially fatal lung edema—so-called pink foam syndrome in elephants.[11,16,21] Potent opioids are also not available in many countries, and avoiding the use of potent opioids mitigates some of the risks posed by these drugs to the management team.[21]

In captive elephants, α_2 adrenoceptor agonists, such as xylazine, medetomidine and detomidine, are often used alone or combined with butorphanol to induce standing sedation.[7,18,20,23,35] The use of xylazine alone has been shown to provide "fair" sedation that is adequate to load animals onto a trailer, whereas its combination with butorphanol provides better sedation and analgesia for minor procedures.[32] Azaperone, a butyrophenone, has also been reported to provide excellent sedation in captive African elephants, although it has no analgesic properties and therefore requires local anesthesia for painful procedures.[32] The use of medetomidine or detomidine in combination with butorphanol has been successfully used to provide adequate standing sedation for minor procedures in African elephants.[23,30]

A new fixed-dose combination consisting of 30 mg/ml butorphanol tartrate, 12 mg/ml azaperone tartrate, and 12 mg/ml medetomidine hydrochloride (BAM, Wildlife Pharmaceuticals [Pty] Ltd, White River, 1240, South Africa) is currently being investigated in a wide variety of southern African wildlife species. Medetomidine produces sedation, muscle relaxation, and analgesia by stimulating α_2-receptors that exist pre- and postsynaptically in tissues throughout the body.[9,10,47] Butorphanol has potent analgesic properties because of agonistic κ receptor activity,[38] whereas azaperone causes sedation and the potentiation of anaesthesia.[21,47] Additionally, azaperone is beneficial in reducing the hypertensive effects of opioids and α_2-agonists because of its α_1-receptor antagonistic activity that results in peripheral vasodilation and bradycardia.[21,25,26,31,47] In combination, these drugs have proven useful for neuroleptanalgesia in wildlife. In South Africa, the successful use of BAM for the complete and reversible immobilization of lions (*Panthera leo*), cheetah (*Acinonyx jubatus*), and blesbok (*Damaliscus pygargus phillipsi*) has already been investigated.[39–41] Internationally, this combination has also been successfully used for complete and reversible immobilization of white-tailed deer (*Odocoileus virginianus*),[5,19,27,28] Rocky Mountain elk (*Cervus canadensis nelsoni*),[51] Nubian ibex (*Capra nubiana*),[22] captive caribou (*Rangifer tarandus granti*),[12] bighorn sheep (*Ovis canadensis*),[44] American beaver (*Castor canadensis*),[34] and black bear (*Ursus americanus*).[52]

The aim of this study was to investigate the effectiveness and physiological effects of BAM for the standing sedation of trained African elephants during routine medical procedures. Antagonism with atipamezole and naltrexone was also investigated.

MATERIALS AND METHODS

The study took place at Camp Jabulani in Kapama Game Reserve (24°25′45.5″S, 31°00′ 50.1″E, altitude 506 m) near Hoedspruit in Limpopo province, South Africa. At the time of the study, Camp Jabulani had a herd of 14 habituated, trained elephants that were previously rescued from across southern Africa and were used for elephant interactions and educational purposes. The elephants were semicaptive, and they were allowed to roam the reserve throughout the day alongside their handlers and then returned to rest and sleep in custom-built enclosures.

The butorphanol, azaperone, and medetomidine fixed-dose combination used in this study was produced by Wildlife Pharmaceuticals. Each milliliter of the solution contained the active pharmaceutical ingredients as 30 mg of butorphanol tartrate, 12 mg of azaperone tartrate, and 12 mg of medetomidine hydrochloride. The animals were sedated on two separate occasions: nine animals during July 2016 and four animals during October 2016. The youngest animal in the herd, a 6-yr-old female, was excluded from the study because she could not successfully be separated from her mother. All the animals were sedated between 8 AM and 1 PM, with environmental temperatures during these periods ranging from 16.7°C to 39.3°C and humidity from 10% to 68%.

Before darting, the shoulder height of each animal was measured with the assistance of each animal's handler. These measurements were used to estimate body weight from shoulder height on the basis of data previously published, as well as data collected over a number of years in the Kruger National Park, South Africa (Malan and Raath, pers. comm.).[8] From these estimated weights, a BAM dose was calculated on the basis of a medetomidine dose of 0.006 mg/kg, which has previously been used in African and Asian elephants.[23,35] A gas-powered dart gun (Pneu-Dart X-Caliber, Pneu-Dart Inc, Palo Alto, CA 94020, USA) and 5-ml darts (14 ga, 7.6-cm-long needle with wire barb, Pneu-Dart Inc) were used to deliver the drug. The entire herd stood alongside each other on an open grass area while each

animal was individually darted, moved away from the herd by its handler, monitored, and returned to the herd after reversal of sedation. Remote darting was done at distances ranging from 10 to 20 m. All injections were administered into the femoral muscle area. To antagonize the effect of the medetomidine, atipamezole (20 mg/ml, Wildlife Pharmaceuticals) at five times the medetomidine dose in milligrams was used. Naltrexone hydrochloride (Trexonil, Wildlife Pharmaceuticals, 50 mg/ml) was used to antagonize butorphanol at 1 mg for each milligram of butorphanol. All the injections were given by hand injection intramuscularly into femoral muscle area in all the elephants. None of the animals exhibited signs of severe agitation or stress while individual counterparts were being sedated and monitored, and all animals returned to the herd safely after reversal.

Monitoring and manipulations of animals

Two stages of induction were timed. Stage I was measured from time of darting until the first signs of sedation. First signs included slowing of ear and tail movement (as sedation deepens, movement ceases), relaxation and "dropping" of the ears forward, sonorous breathing, protrusion of the penis from the prepuce, relaxation of the trunk and its resting on the ground, widening of the stance, and ataxia.[11] Stage II was measured from time of darting until monitoring could be safely started, at which point, the darted animal had been walked away from the herd to stand completely still in a shaded area while all the monitoring equipment was attached.

Most animals could safely be monitored within 25–35 min after darting, although one older bull exhibited initial excitement at first signs of sedation and subsequently took longer to stand still enough to be safely monitored. Monitoring of this animal only started at 45 min after darting, but no additional drugs were required to handle this animal safely. During monitoring, physiological parameters were recorded every 5 min for 25–30 min. Heart rate (HR) was measured manually by palpation of the pulse in the auricular artery. Respiratory frequency (f_R) was measured by counting exhaled breaths. Peripheral oxygen saturation (SpO_2) was measured with a pulse oximeter (Nonin PalmSAT 2500, Amsterdam, 1075, The Netherlands) attached to the ear at the same location on all the elephants. Rectal temperature was measured by means of a hand-held digital thermometer (Checktemp 1, Hanna Instruments [Pty] Ltd, Woonsocket, Providence County, RI 02895, USA) inserted into the rectum and pressed against the intestinal wall. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) was measured by means of invasive intra-arterial measurements at the auricular artery (arteria auricularis) with an invasive blood pressure monitor (IntraTorr, Meyer & Salter, Johannesburg, 2000, South Africa) connected to a Deltran II pressure transducer (Utah Medical, Midvale, UT 84047, USA). This instrument also reliably measured heart rate, thereby serving as a cross-reference to the pulse oximeter and manual measurements. The auricular artery was used to collect arterial blood samples from each animal. Three arterial blood samples were collected from each animal at 0, 10, and 20 min of monitoring. The puncture was done anaerobically with a heparinized syringe with an 18-ga needle. Blood sample analysis was conducted immediately with a portable analyzer and cartridges (i-STAT® cartridges CG4+, CHEM8+, Abaxis, Union City, CA 94587, USA). Variables measured included pH, partial pressure of arterial oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), lactate, hematocrit, sodium, potassium, chloride, urea, creatinine, glucose, and ionized calcium levels. Actual base excess, actual bicarbonate, oxygen saturation, and hemoglobin were calculated automatically from the measured values.

After monitoring was completed and all the monitoring equipment was removed, the animal was given the reversal and walked back to the herd. Antagonists were injected intramuscularly into the femoral muscle area, and the time elapsed from injection until the first signs of recovery (voluntary trunk and ear movements) were recorded.

Statistical analysis

For the analysis of anesthetic effects, the elephant's HR, rectal temperature, f_R, SBP, DBP, MAP, SpO_2, PaO_2, $PaCO_2$, pH, and lactate were measured, and the area under the curve (AUC) was calculated by a trapezoid method for every measurement for the immobilization period (30 min). The mean AUC were used as response variables in linear regression models. The bodyweight (estimated from measured shoulder height), shoulder height, and dose (ml) were used as continuous explanatory variables. Gender was included in models to control for a possible confounding effect. For evaluation of f_R effect on PaO_2 and SpO_2, linear regression models with PaO_2 or SpO_2 mean AUC as

Table 1. BAM doses for the standing sedation of African elephant (*Loxodonta africana*). Doses are given as mean ± SD (range).

Dose	BAM (ml)	Butorphanol (mg)	Azaperone (mg)	Medetomidine (mg)
Total dose	1.66 ± 0.51 (1.0–2.7)	49.88 ± 15.41 (30.0–81.0)	19.95 ± 6.17 (12.0–32.4)	19.95 ± 6.17 (12.0–32.4)
Dose/cm shoulder height	0.006 ± 0.001 (0.005–0.009)	0.19 ± 0.04 (0.14–0.26)	0.076 ± 0.015 (0.055–0.102)	0.076 ± 0.015 (0.055–0.102)
Estimated dose/kg	0.0005 ± 0.0001 (0.0004–0.0007)	0.016 ± 0.002 (0.013–0.02)	0. 006 ± 0.0008 (0.005–0.008)	0. 006 ± 0.0008 (0.005–0.008)

response variables and f_R as an explanatory variable were used. For evaluation of the elephant's HR, rectal temperature, f_R, SBP, DBP, MAP, SpO_2, PaO_2, $PaCO_2$, pH, and lactate over time, monitoring times were assigned as either 20–30 min, 30–40 min, 40–50 min, or 50–60 min after darting. Responses over time intervals were analyzed by a restricted maximum likelihood estimation (REML) model. Data analysis was performed by the Variance Estimation and Precision module of Statistica 13.5.0 software program (TIBCO, Palo Alto, CA 94020, USA). For analysis of the effect of BAM on induction (time to first sign of sedation) and recovery time, linear regression models were used. Normality was verified by scatter and normality plots of standardized residuals. For all statistical analyses, Statistica 13.5.0 software was used; $P \leq 0.05$ were considered statistically significant. Data are reported as mean ± SD (range). The Kolmogorov–Smirnov and Lilliefors test for normality showed that physiological data were not normally distributed, and this data is presented as median ± SD (interquartile range).

RESULTS

In total, seven females (ages 6–31 yr) and six males (ages 9–35 yr) were sedated (mean 19 ± 10 yr). Estimated elephant weights ranged from 1,750 to 5,250 kg (mean 3,140 ± 1,166 kg). The smallest elephant in the study group was an 8-yr-old female, and the largest was a 35-yr-old male.

In all 13 monitored animals, complete sedation occurred after a single administration of BAM. The doses used are given in Table 1.

First signs of sedation were observed within 3–10 min (mean 6 ± 2 min) after darting, and monitoring of the animals started on average at 24 ± 9 min after darting. A weak association was found between the BAM dose (ml) and first sign of sedation ($P = 0.04$; $R^2 = -0.22$) so that higher BAM doses resulted in faster induction times. All inductions observed were calm and smooth, although the second largest bull in the herd (age 27 yr; 310 cm shoulder height; estimated weight 4,900 kg) showed initial excitation at the first signs of sedation and proceeded to run away when its handler tried to walk it away from the herd for monitoring. The handler managed to calm it down, and it could eventually be monitored safely at 45 min after darting for the complete monitoring period of 25–30 min. Hypersalivation was observed in four of the elephants. None of the elephants showed reaction to minor procedures such as blood sampling, vaccination, or microchipping. One elephant required treatment of an abscess and showed no response to laceration, draining, and flushing of the abscess. None of the elephants became recumbent or responsive during monitoring.

Arterial blood gas values were at acceptable levels throughout sedation: mean $PaO_2 = 83.0 \pm 10.64$ mm Hg; mean $PaCO_2 = 48.0 \pm 6.32$ mm Hg. There was no association between f_R and PaO_2 ($P = 0.226$) or f_R and SpO_2 ($P = 0.836$). Table 2 presents the median ± SD and range of the main parameters measured during monitoring and the results of the blood gas analyses. None of the physiological parameters measured differed over the monitoring periods ($P > 0.05$), except for PaO_2, which was significantly lower ($P = 0.024$) at 20–30 min after darting (73.4 ± 11.52 mm Hg) compared with 40–50 min after darting (84.8 ± 5.79 mm Hg).

The average time from darting until the reversal was injected was 57.7 ± 9.07 min. Recovery was smooth and calm in all animals. Time from injection until the first signs of recovery was 4.6 ± 2.01 min (range 1–8 min). There was no association between time to first sign of recovery and the BAM dose (ml, $P > 0.05$). The average time elapsed from darting until monitoring was complete was 51.5 ± 9.01 min. No mortalities occurred during the study, and no subsequent adverse effects were noted.

Table 2. The physiological response of elephants to standing sedation with a butorphanol-azaperone-medetomidine fixed-dose combination (BAM) over a 25–30-min period.

		Time after darting				
Variable[a]	Unit	20–30 min	30–40 min	40–50 min	50–60 min	Overall (range)
HR	beats/min	40.0 ± 6.94	38.0 ± 6.45	42.0 ± 7.38	38.5 ± 5.38	40.0 ± 6.55 (35–44)
f_R	breaths/min	6.0 ± 1.6	6.0 ± 1.91	6.0 ± 1.65	6.0 ± 1.47	6.0 ± 1.67 (5–8)
SpO_2	%	100.0 ± 1.93	100.0 ± 1.32	99.0 ± 1.62	99.0 ± 1.38	99.5 ± 1.62 (98–100)
T	°C	36.6 ± 0.48	35.8 ± 0.57	36.4 ± 0.39	36.3 ± 0.6	36.3 ± 0.53 (35.8–36.5)
SP	mm Hg	122.0 ± 22.84	115.0 ± 18.32	114.0 ± 20.48	119.0 ± 26.13	118.5 ± 20.22 (107–131)
DP	mm Hg	80.0 ± 20.07	87.5 ± 13.78	84.0 ± 15.39	86.0 ± 13.81	86.0 ± 15.0 (77–95)
MAP	mm Hg	95.0 ± 20.47	94.0 ± 14.26	92.0 ± 17.3	97.0 ± 17.3	94.5 ± 16.12 (88–108)
PaO_2	mm Hg	78.0 ± 11.53	83.0 ± 10.72	85.5 ± 5.79	87.0 ± 7.1	83.0 ± 10.64 (75–87)
$PaCO_2$	mm Hg	51.0 ± 5.77	49.7 ± 7.99	47.0 ± 5.72	44.0 ± 1.74	48.0 ± 6.32 (45–54)
Lactate	mmol/L	0.8 ± 0.28	0.77 ± 0.24	0.73 ± 0.28	0.74 ± 0.28	0.74 ± 0.25 (0.61–0.91)
pH		7.37 ± 0.03	7.36 ± 0.07	7.37 ± 0.04	7.36 ± 0.04	7.36 ± 0.05 (7.34–7.38)

[a] HR indicates heart rate; f_R, respiratory frequency; SpO_2, peripheral capillary oxygen saturation; T, rectal temperature; SP, systolic pressure; DP, diastolic pressure; MAP, mean arterial pressure; PaO_2, partial pressure of oxygen; $PaCO_2$, partial pressure of carbon dioxide.

DISCUSSION

Published reports on the standing sedation of African elephants are limited.[23,30,42] The doses used in the current study were based on ranges similar to those reported for medetomidine and butorphanol in elephants, although the azaperone dose was very low compared with previously reported ranges of 0.068–0.107 mg/kg.[32] Some of the earliest reports on standing sedation of captive and trained African elephants used only azaperone.[42] It was noted that the ideal neuroleptic to achieve standing sedation in this species should have a wide margin of safety, be as small an injection volume as possible, produce consistently predictable results, have a rapid induction and a recovery within 1–2 hr, and should sedate the animal enough for minor procedures and handling while still maintaining the elephant in a standing position without excessive restraint.[42] The results from the current study indicate that the BAM combination possesses all these qualities. Injection volumes did not exceed 3 ml, physiological parameters were consistently stable throughout sedation, sedation was observed within 10 min and animals could be safely monitored within 25 min after darting, and recovery commenced within 5 min after injection of the antagonists. Additionally, none of the animals became unstable or recumbent, and none of the elephants required additional drugs to maintain sedation throughout monitoring.

Similar induction times have been reported as in the current study with a combination of butorphanol (0.03 ± 0.007 mg/kg) and medetomidine (0.009 ± 0.002 mg/kg) in captive African elephants.[23] However, it was reported that a supplemental injection of 0.003 ± 0.001 mg/kg medetomidine and 0.007 ± 0.003 mg/kg butorphanol was required for 11 out of 33 of the sedations. Induction times were also reduced with the addition of hyaluronidase (1,000–2,000 IU) during eight sedations. The addition of hyaluronidase to BAM may improve induction times, as well, and should be further investigated. The use of detomidine (0.013–0.020 mg/kg) and butorphanol (0.013–0.020 mg/kg) in three African elephants requiring 14 standing sedation procedures has also been reported.[30] First signs of sedation were observed within 11.6 ± 5.9 min. This initial sign of sedation was ranked as "mild" in four of the sedation procedures, and these animals received subsequent supplemental doses. Two additional sedation events that required prolonged procedures also received supplemental doses to extend sedation and analgesia. For seven of the eight procedures not requiring supplemental doses, maximal drug effect appeared to occur at 25–30 min after injection.[30] These results are similar to those observed in the current study, although none of the animals in the current study required supplemental doses to maintain sedation, which suggests an advantage of BAM over detomidine-butorphanol. This result may in part be a result of the addition of azaperone in the BAM combination, resulting in the potentiation of sedation, as well as the high selectivity of medetomidine for α_2 receptors compared with detomidine.[43,47]

Medetomidine is a potent α_2 adrenoreceptor agonist, with specific actions at the receptors responsible for sedation and analgesia.[6,9,37,49] Al-

though it is commonly used as a sedative in veterinary medicine, bradycardia and hypotension are inherent side effects that have been reported.[10] Medetomidine causes peripheral vasoconstriction by activating postsynaptic α_{2B} adrenoceptor subtypes in the vascular smooth muscle.[43,49] As a result, an initial increase in blood pressure may be seen because of an increase in systemic vascular resistance, followed by a decrease in blood pressure from the baroreceptor reflex and the suppression of cardiac output.[29,36] No bradycardia was observed in any of the elephants treated with BAM. The heart rates of conscious, standing African and Asian elephants has been reported to be 24–55 beats/min.[11,15] A MAP of 100–191 mm Hg, SBP of 138–239 mm Hg, and DBP of 70–164 mm Hg has also been reported in conscious, standing elephants.[15] Most of the animals in the current study were mildly hypotensive, although one elephant, a 29-yr-old male with an estimated weight of 4,550 kg, was moderately hypotensive throughout sedation with a mean systolic/diastolic (mean) blood pressure of 100/73 (82). However, blood pressure was not low enough to warrant reversal, and it is unclear what caused this mild to moderate hypotension. Cardiovascular side effects that have been reported in other species with the use of medetomidine include transient hypertension and an increase in vascular resistance during the loading phase, bradycardia, and a reduction in cardiac output followed by hypotension.[43,50] This pattern of effect was not noted, with no initial hypertension observed in any of the animals. Azaperone is a butyrophenone that causes peripheral vasodilation by blocking the α_1 adrenergic receptors and inducing vasodilation in striated muscle arterioles, thereby reducing vascular resistance and reducing blood pressure.[24,33] Azaperone is often included in opioid-based immobilization protocols in large species, such as elephant, to counteract the hypertensive effects of the opioids.[13,14] The inclusion of azaperone in the BAM combination may have contributed to the mild hypotension observed but was likely not the predominant cause because comparatively low doses were used compared with doses reported for African elephants.[33]

Rectal temperatures fell within acceptable ranges reported for elephants.[11] Respiratory parameters were stable in all the animals throughout sedation and fell within the normal ranges reported for conscious, standing elephants.[11,15,50] In conscious, standing African elephants, PaO_2 and $PaCO_2$ values of 96.21 ± 1.55 and 44.19 ± 0.48 mm Hg, respectively, have been reported in conscious, standing African elephants, whereas in Asian elephants, PaO_2 and $PaCO_2$ values of 103 ± 2 and 39.4 ± 0.3 mm Hg, respectively, have been reported.[15,17] In the current study, only one elephant had clinically significant hypoxemia characterized by a $PaO_2 < 60$ mm Hg.[46] This elephant was also hypercapnic ($PaCO_2 > 50$ mm Hg), although pH and SpO_2 fell within acceptable ranges. The former indicates mild hypoventilation, although the animal had f_R values ranging from 6 to 9 breaths/min. Hypoventilation in white-tailed deer immobilized with butorphanol, azaperone, and medetomidine has been reported and attributed to the respiratory depressant effect of both butorphanol and medetomidine.[27] It must be noted that doses of 0.43 mg/kg butorphanol, 0.36 mg/kg azaperone, and 0.14 mg/kg medetomidine were used, which were significantly higher than those used in the elephants. Butorphanol is a synthetic opioid that is centrally acting with mixed agonist-antagonistic activity at μ-opioid receptors and agonistic activity at κ-opioid receptors.[20] Although it is less likely to induce respiratory depression compared with pure μ agonists,[1,48] its combination with medetomidine may adversely affect respiration because of their synergistic effect. α_2 Agonists have been reported to have negative effects on respiration because of the significant decrease in oxygen delivery as a result of a drop in cardiac output.[2–4] This outcome would likely also explain the generally lower PaO_2 values of the remaining group of elephants in the current study compared with the values reported for fully conscious elephants.

Recoveries in all the animals were smooth and uneventful, and animals could be moved back to the remainder of the herd without any observable stress elicited in either the individual or the rest of the herd. Recoveries were quicker than reported when yohimbine was used to antagonize detomidine.[30] This result was probably due to the use of atipamezole in the current study, because atipamezole has the highest α_2 receptor affinity of all the available α_2 antagonists and is therefore preferred for antagonizing medetomidine.[43] Slower recoveries compared with the current results have also been reported with the use of a lower atipamezole : medetomidine ratio.[22]

CONCLUSIONS

Overall, BAM produced a good level of sedation in all the animals. Inductions and recoveries were uneventful and quick, and all animals could safely be handled throughout the monitoring period without the need for additional doses. The butor-

phanol in the combination provided adequate analgesia for minor procedures, such as vaccinations and wound treatments, and none of the animals exhibited any signs of discomfort. Although all the animals were mildly hypotensive, physiological parameters were stable throughout monitoring and indicated safe sedation of all the animals. The use of this combination can be recommended in captive, trained African elephants at a dose of 0.006 ± 0.001 ml/cm shoulder height. Reversal with intramuscular naltrexone at a dose of 1 mg/mg butorphanol and atipamezole at a dose of 5 mg/mg medetomidine is also recommended.

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LITERATURE CITED

1. Bush M, Citino SB, Lance WR. The use of butorphanol in anesthesia protocols for zoo and wild mammals. In: Miller RE, Fowler M. (eds.). Zoo and wild animal medicine, Volume 7, Current therapy. St Louis (MO): Elsevier; 2011. p. 1–9.

2. Caulkett NA, Cribb PH, Duke T. Cardiopulmonary effects of medetomidine-ketamine immobilization with atipamezole reversal and carfentanil-xylazine immobilization with naltrexone reversal: a comparative study in domestic sheep (*Ovis ovis*). J Zoo Wildl Med. 1994;25(3):376–389.

3. Caulkett NA, Duke T, Cribb PH. Cardiopulmonary effects of medetomidine : ketamine in domestic sheep (*Ovis ovis*) maintained in sternal recumbency. J Zoo Wildl Med. 1996;27(2):217–226.

4. Chittick E, Horne W, Wolfe B, Sladky K, Loomis M. Cardiopulmonary assessment of medetomidine, ketamine and butorphanol anaesthesia in captive Thomson's gazelles (*Gazella thomsoni*). J Zoo Wildl Med. 2001;32(2):168–175.

5. Cook W, Cain D, Hensley T. Tissue residue levels of butorphanol, azaperone, medetomidine, atipamezole, and naltrexone in white-tailed deer (*Odocoileus virginanus*) at 11 and 21 days post intramuscular injection. Poult Fish Wildl Sci. 2016;4(2):10–11.

6. Cullen LK. Medetomidine sedation in dogs and cats: a review of its pharmacology, antagonism and dose. Br Vet J. 1996;152:519–535.

7. Dangolla A, Silva I, Kuruwita VY. Neuroleptanalgesia in wild Asian elephants (*Elephas maximus maximus*). Vet Anaesth Analg. 2004;31(4):276–279.

8. du Toit JG. Veterinary care of African elephants. Johannesburg (South Africa): South African Veterinary Foundation/Novartis Wildlife Fund; 2001.

9. Fahlman Å. Advances in wildlife immobilisation and anaesthesia. PhD Dissertation, 2008. Swedish Univ. of Agricultural Sciences, Uppsala (Sweden).

10. Flacke JW. Alpha 2-adrenergic agonists in cardiovascular anesthesia. J Cardiothorac Vasc Anesth. 1992;6(3):344–359.

11. Fowler ME, Mikota SK. Biology, medicine and surgery of elephants. Ames (IA): Blackwell Publishing; 2006.

12. Hansen CM, Beckmen KB. Butorphanol-azaperone-medetomidine for the immobilization of captive caribou (*Rangifer tarandus granti*) in Alaska, USA. J Wildl Dis. 2018;54(3):650–652.

13. Hattingh J, Knox CM, Raath JP. Arterial blood pressure and blood gas composition of white rhinoceroses under etorphine anaesthesia. S Afr J Wildl Res. 1994;24:12.

14. Hattingh J, Knox CM, Raath JP, Keet DF. Arterial blood-pressure in anesthetized african elephants. S Afr J Wildl Res. 1994;24(1–2):15–17.

15. Honeyman VL, Pettifer GR, Dyson DH. Arterial blood pressue and blood gas values in normal standing and laterally recumbent African (*Loxodonta africana*) and Asian (*Elaphus maximus*) elephants. J Zoo Wildl Med. 1992;23(2):205–210.

16. Hyeroba D, Apell P, Goldberg T, Shafer LA, Kidega T, Asimwe C. Ketamine-medetomidine regimen for chemical immobilisation of free-ranging chimpanzees (*Pan troglodytes schweinfurthii*) in Uganda. Vet Rec. 2013;172(18):475.

17. Isaza R, Behnke BJ, Bailey JK, McDonough P, Gonzalez NC, Poole DC. Arterial blood gas control in the upright versus recumbent Asian elephant. Respir Physiol Neurobiol. 2003;134(2):169–176.

18. Jacobson ER, Allen J, Martin H, Kollias GV. Effects of yohimbine on combined xylazine-ketamine–induced sedation and immobilization in juvenile African elephants. Am Vet Med Assoc. 1985;187(11):1195–1198.

19. Kirschner SM, Rodenkirch R. Assessment of butorphanol-azaperone-medetomidine combination as anesthesia for semen collection and evaluation of semen quality in white-tailed deer (*Odocoileus virginianus*). Anim Reprod Sci. 2017;184:196–203.

20. Knych HK, Casbeer HC, Mckemie DS, Arthur RM. Pharmacokinetics and pharmacodynamics of butorphanol following intravenous administration to the horse. J Vet Pharmacol Ther. 2013;36(1):21–30.

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

22. Lapid R, Shilo-Benjamini Y. Immobilization of captive Nubian ibex (*Capra nubiana*) with butorphanol-midazolam-medetomidine or butorphanol-azaperone-medetomidine and atipamezole reversal. J Zoo Wildl Med. 2015;46(2):291–297.

23. Lüders I, Tindall B, Young D, van der Horst G, Botha S, Luther I, Maree L, Bertschinger HJ. Standing sedation with medetomidine and butorphanol in captive African elephants (*Loxodonta africana*). Vet J. 2016; 209:190–192.

24. Mentaberre G, López-Olvera JR, Casas-Díaz E, Fernández-sirera L, Marco I, Lavín S. Effects of azaperone and haloperidol on the stress response of drive-net captured Iberian ibexes (*Capra pyrenaica*). Eur J Wildl Res. 2010;56(5):757–764.

25. Mentaberre G, Lopez-Olvera JR, Casas-Díaz E, Marco I, Lavín S. Haloperidol and azaperone in drive-net captured southern chamois (*Rupicapra pyrenaica*). J Wildl Dis. 2010;46(3):923–928.

26. Meyer LCR, Hetem RS, Fick LG, Matthee A, Mitchell D, Fuller A. Thermal, cardiorespiratory and cortisol responses of impala (*Aepyceros melampus*) to chemical immobilisation with 4 different drug combinations. S Afr Vet Assoc. 2008;79(3):121–129.

27. Mich PM, Wolfe LL, Sirochman TM, Sirochman MA, Davis TR, Lance W, Miller MW. Evaluation of intramuscular butorphanol, azaperone, and medetomidine and nasal oxygen insufflation for the chemical immobilization of white-tailed deer, *Odocoileus virginianus*. J Zoo Wildl Med. 2008;39(3):480–487.

28. Miller BF, Osborn DA, Lance WR, Howze MB, Warren RJ, Miller KV. Butorphanol-azaperone-medetomidine for immobilization of captive white-tailed deer. J Wildl Dis. 2009;45(2):457–467.

29. Murrell JC, Hellebrekers LJ. Medetomidine and dexmedetomidine: a review of cardiovascular effects and antinociceptive properties in the dog. Vet Anaesth Analg; 2005;32(3):117–127.

30. Neiffer DL, Miller MA, Weber M, Stetter M, Fontenot DK, Robbins PK, Pye GW. Standing sedation in African elephants (*Loxodonta africana*) using detomidine-butorphanol combinations. J Zoo Wildl Med. 2005;36(2):250–256.

31. Portas TJ. A review of drugs and techniques used for sedation and anaesthesia in captive rhinoceros species. Aust Vet J. 2004;82(9):542–549.

32. Ramsay E. Standing sedation and tranquilization in captive African elephants (*Loxodonta africana*). Proceedings of the American Association of Zoo Veterinarians. 2000. p. 111–114.

33. Riviere JE, Papich MG (eds.). Veterinary pharmacology and therapeutics. 9th ed. Ames (IA): Wiley-Blackwell; 2009.

34. Roug A, Talley H, Davis T, Roueche M, DeBloois D. A mixture of butorphanol, azaperone, and medetomidine for the immobilization of American beavers (*Castor canadensis*). J Wildl Dis. 2018;54(3): 617–621.

35. Sarma B, Pathak SC, Sarma KK. Medetomidine—a novel immobilizing agent for the elephant (*Elephas maximus*). Res Vet Sci. 2002;73(3):315–317.

36. Savola JM. Cardiovascular actions of medetomidine and their reversal by atipamezole. Acta Vet Scand. 1989;85:39–47.

37. Scheinin H, Virtanen R, MaDonald E, Lammintausta R, Scheinin M. Medetomidine—a novel α_2-adrenoceptor agonist: a review of its pharmacodynamic effects. Prog Neuropsychopharmacol Biol Psychiatry. 1989;13:635–651.

38. Schnellbacher R. Butorphanol. J Exot Pet Med. 2010;19(2):192–195.

39. Semjonov A, Andrianov V, Raath JP, Orro T, Laubscher L, Pfitzer S, Tiirats T. Evaluation of butorphanol-azaperone-medetomidine (BAM) in captive blesbok immobilization (*Damaliscus pygargus phillipsi*). Vet Anaesth Analg. 2018;45(4):496–501.

40. Semjonov A, Andrianov V, Raath JP, Orro T, Laubscher L, Pfitzer S, Tiirats T, Rogers PS. Evaluation of butorphanol-azaperone-medetomidine (BAM) in captive cheetah (*Acinonyx jubatus*) immobilization. Vet Anaesth Analg. 2019;46(1):90–95.

41. Semjonov A, Andrianov V, Raath JP, Orro T, Venter D, Laubscher L, Pfitzer S. Evaluation of BAM (butorphanol–azaperone–medetomidine) in captive African lion (*Panthera leo*) immobilization. Vet Anaesth Analg. 2017;44(4):883–889.

42. Silberman MS. Tranquilization of the African elephant (*Loxodonta africana*, Blumenbach) with the neuroleptic azaperone. J Zoo Anim Med. 1977;8(4):7–8.

43. Sinclair MD. A review of the physiological effects of alpha 2-agonists related to the clinical use of medetomidine in small animal practice. Can Vet J. 2003;44:885–897.

44. Smith JB, Jenks JA, Grovenburg TW, Klaver RW. Disease and predation: sorting out causes of a bighorn sheep (*Ovis canadensis*) decline. PLoS One. 2014;9(2):e88271.

45. Stegmann GF. Etorphine-halothane anaesthesia in two five-year-old African elephants (*Loxodonta africana*). J S Afr Vet Assoc. 1999;70(4):164–166.

46. Still J, Raath JP, Matzner L. Respiratory and circulatory parameters of African elephants (*Loxodonta africana*) anaesthetised with etorphine and azaperone. J S Afr Vet Assoc. 1996;67(3):123–127.

47. Swan GE. Drugs used for the immobilization, capture, and translocation of wild animals. In: McKenzie AA (ed.). The capture and care manual. Pretoria (South Africa): The South African Veterinary Foundation; 1993. p. 3–59.

48. Tana LM, Isaza R, Koch DE, Hunter RP. Pharmacokinetics and intramuscular bioavailability of a single dose of butorphanol in Asian elephants (*Elephas maximus*). J Zoo Wildl Med. 2010;41(3):418–425.

49. Virtanen R, Savola JM, Saano V, Nyman L. Characterization of the selectivity, specificity and potency of medetomidine as an alpha 2-adrenoceptor agonist. Eur J Pharmacol. 1988;150(1–2):9–14.

50. West G, Heard DJ, Caulkett NA (eds.). Zoo animal and wildlife immobilization and anesthesia. 1st ed. Ames (IA): Blackwell Publishing; 2007.

51. Wolfe LL, Fisher MC, Davis TR, Miller MW. Efficacy of a low-dosage combination of butorphanol, azaperone, and medetomidine (BAM) to immobilize Rocky Mountain elk. J Wildl Dis. 2014;50(3):676–680.

52. Wolfe LL, Goshorn C, Baruch-Mordo S. Immobilization of black bears (*Ursus americanus*) with a combination of butorphanol, azaperone, and medetomidine. J Wildl Dis. 2008;44(3):748–752.

53. Young E. Notes on the chemical immobilization and restraint of Addo elephant (*Loxodonta africana*). Koedoe. 1972;15:97–99.

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