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Research Article

COMPARISON OF THIAFENTANIL-AZAPERONE AND ETORPHINE-AZAPERONE FOR THE IMMOBILIZATION OF FREE-RANGING BLACK RHINOCEROS (*DICEROS BICORNIS*)

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Abstract: The efficacy, safety, and cardiorespiratory effects of two immobilizing drug combination—etorphine, azaperone, and hyaluronidase (EA) and thiafentanil, azaperone, and hyaluronidase (TA)—were evaluated and compared in free-ranging black rhinoceros (*Diceros bicornis*). To improve the safety of captures and animal welfare, we aimed to determine whether one drug combination offered advantages in terms of safety, efficacy, and physiological stability during immobilization. In a randomized, prospective field study, 12 male and 12 female adult black rhinoceroses were randomly immobilized with either EA ($n = 12$) or TA ($n = 12$), with doses based on rhinoceros age and sex. Drugs were administered IM via remote darting. Induction, immobilization, and recovery times were recorded and compared. RR, HR, rectal temperature, oxygen saturation, and blood pressure were measured every 5 min for 30 min and arterial blood gases were measured every 10 min. Rhinoceroses in the TA treatment group had faster induction times (2.88 ± 0.75 min) than those in the EA group (3.95 ± 0.77 min). Both treatments resulted in decreases in HR and blood pressure over time. Both treatments resulted in reduced partial pressure of arterial oxygen (hypoxemia) and increased partial pressure of carbon dioxide in arterial blood (hypercapnia), with no differences between groups. Increased alveolar-arterial oxygen gradient, acidemia, decreased base excess, hypercapnia, and hyperlactatemia were observed, indicating respiratory and metabolic lactic acidosis in both groups. Both treatments provided effective induction, immobilization, and recovery, although hypoxemia, hypercapnia, and acidosis were present. Either combination can be used successfully for immobilizing black rhinoceros. However, the faster induction induced by TA may reduce stress responses and overexertion, potentially lowering associated risks.

INTRODUCTION

Before the 1960s, wildlife capture, including that of rhinoceroses, was primarily achieved through physical methods using ropes, traps, and vehicles. These techniques posed significant risks to both the rhinoceroses and the operators.[39,47] The introduction of chemical immobilization after 1960 revolutionized wildlife management by providing a safer, more controlled alternative. This method involves the administration of drugs that induce a reversible depression of the CNS, suppressing motor, sensory, and autonomic reflexes. As a result, chemical immobilization facilitates the handling of an animal and the performance of minor procedures with less stress to the animal.[28] Remote delivery devices also evolved, significantly enhancing the efficiency and safety of wildlife immobilization. Modern darting systems, equipped with robust needles capable of penetrating thick skin, have contributed to a dramatic reduction in mortality rates associated with capture.[22]

Effective immobilizing wildlife drug combinations must be potent for small-volume delivery, have a rapid onset to minimize capture-related stress (e.g., exertion, hyperthermia and acidosis), and be fully reversible for safe recovery.[17] Etorphine has long been a cornerstone of black rhinoceros (*Diceros bicornis*) immobilization, first described in 1965 by King.[21] Early use of etorphine, often in combination with different tranquilizers, demonstrated good potency, allowing for smaller drug volumes and safer, more precise administration.[9,45] Subsequent to

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this initial work, other drug combinations, such as etorphine with acetylpromazine, were reported to provide effective immobilization. However, challenges such as prolonged induction times and complications from secondary factors such as environmental hazards persisted.[3,9,20,24] The inclusion of hyaluronidase, which liquefies the ground substance between cells (hyaluronic acid) and thereby enhances tissue permeability and increases the bioavailability of coadministered drugs following IM injection, further improved the induction process and reduced stress, marking significant advances in the late 20th century.[23,37] In recent years, the inclusion of newer drugs such as azaperone, detomidine, medetomidine, and midazolam have become increasingly popular for use in rhinoceros species due to their rapid onset, muscle-relaxant properties, and their synergistic effects, whereas etorphine continues to be the primary immobilizing agent.[1,6,12,27]

Thiafentanil is a newer addition to the market of potent opioids used for wildlife immobilization, offering an alternative to long-established agents such as etorphine. Developed for its rapid onset and shorter duration of action compared with other opioids, thiafentanil has gained popularity for use in scenarios requiring quick and efficient chemical restraint.[1] However, the use of thiafentanil is not without challenges. Like etorphine, thiafentanil can also cause significant adverse effects, including cardiorespiratory compromise, muscle rigidity, gastrointestinal immobility (leading to bloat in ruminants), and disruption of thermoregulation. These issues may contribute to hypoxemia, hypercapnia, metabolic disturbances, capture myopathy, and increased risk of morbidity or mortality.[30,44]

As a critically endangered species according to the International Union for Conservation of Nature,[10] black rhinoceros often require chemical immobilization for essential activities such as relocation, medical treatment, DNA collection, research, population management, and dehorning to deter poaching. The use of potent opioids delivered IM via projectile dart is common practice for these procedures. Although the use of etorphine is well documented for immobilizing both black and white rhinoceroses (*Ceratotherium simum*),[24,52] thiafentanil's effects are less studied and no published comparisons exist between etorphine and thiafentanil in black rhinoceros. Studies on thiafentanil in antelope suggest it may produce a faster induction compared with etorphine, with similar cardiorespiratory effects,[43,44] but whether this is true in black rhinoceros is not known. Furthermore, although the use of thiafentanil is generally avoided in white rhinoceros, it has been suggested that black rhinoceros may exhibit a lower sensitivity to opioid-induced cardiorespiratory compromise, highlighting the need for species-specific investigations into its safety and efficacy in this species.[1]

The aim of this study was to evaluate the safety and efficacy of two drug combinations—etorphine-azaperone-hyaluronidase (EA) and thiafentanil-azaperone-hyaluronidase (TA)—during planned dehorning events in free-ranging black rhinoceros. Data on induction, immobilization, and recovery times, as well as cardiopulmonary effects, were collected and analyzed to determine whether one combination offered safer, more effective immobilization, thereby improving animal welfare and ensuring the safety of personnel during capture and handling.

MATERIALS AND METHODS

Animals and housing

This study was approved by the University of Pretoria Research and Animal Ethics Committees (REC059-21) and the Wildlife Pharmaceuticals Animal Ethics Committee (WPAEC-2021-RHINO-46-B). Twenty-four free-ranging black rhinoceroses were included in the study, all of which were immobilized for routine annual horn trimming (dehorning). The animals selected for the study were either estimated or known to be >3.5 yr. In cases where age was unknown, it was estimated based on body size, height, and if weaned.[11] The rhinoceroses were classified as E-class (3.5–7 yr) or F-class (>7 yr) according to their age.[11] Nine E-class (subadults) and 15 F-class (adults) rhinoceroses were selected.

The study population included 12 males (bulls) and 12 females (cows and heifers), with the E-class group composed of 4 cows and 5 bulls and the F-class group consisting of 8 cows and 7 bulls. Each rhinoceros was assigned a body condition score (BCS) by assessing seven body regions on a 5-point scale.[48] Only animals with a BCS of ≥3 (indicating fair-to-excellent condition) were included in the study.

The study was conducted in two associated private nature reserves adjacent to Kruger National Park in South Africa. These reserves are in the Limpopo and Mpumalanga provinces, at elevations between 150 and 600 m above sea level.

Study design

The research was designed as a prospective, unpaired, randomized, and blinded study comparing the effects of the two immobilization drug combinations. Blinding was ensured as the investigator on the ground remained unaware of the

drug combination administered until all data collection and processing of the rhinoceros had been completed. One group of rhinoceroses received a combination of etorphine (Captivon®, 9.8 mg/ml, Wildlife Pharmaceuticals (Pty) Ltd., White River, 1240, South Africa), azaperone (100 mg/ml, V-Tech, Midrand, 1685, South Africa), and hyaluronidase (5,000 IU, Kyron Laboratories, Johannesburg, 2094, South Africa) (EA treatment group), whereas the other group received thiafentanil (Thianil®, 10 mg/ml, Wildlife Pharmaceuticals (Pty) Ltd.), azaperone, and hyaluronidase (TA treatment group). Times to induction, immobilization, and recovery were recorded. Each rhinoceroses' recovery quality was assessed after administration of the opioid antagonist naltrexone (Trexonil®, naltrexone hydrochloride, 50 mg/ml, Wildlife Pharmaceuticals (Pty) Ltd.).[43]

Immobilization and monitoring

Before immobilization, animals were randomly assigned to either the EA or the TA treatment group by the veterinarian responsible for darting. Both groups were antagonized with naltrexone, with EA rhinoceroses receiving 20 times the etorphine dose and TA rhinoceroses receiving 10 times the thiafentanil dose on a milligram-per-milligram basis, based on the most recent literature.[1,42]

Etorphine and thiafentanil doses were determined based on the age, sex, and estimated size of each rhinoceros. E-class rhinoceroses received 3.5 mg (heifers) or 4 mg (bulls) of the potent opioid, whereas F-class rhinoceroses received 4 mg (cows) or 4.5 mg (bulls), combined with azaperone (10 mg per 1 mg of potent opioid) and hyaluronidase (5,000 IU). Doses were based on the most recent literature and recommendations.[1,27,36,46]

Once located by fixed-wing aircraft, the rhinoceroses were darted from a helicopter by using a Pneu-Dart 389 projector (Pneu-Dart. Inc., Williamsport, PA 17701, USA) with 2-ml, 2.5-in. (63.5-mm) barbed needle Pneu-Dart darts (C-Type Darts, Pneu-Dart. Inc.) containing the drug combinations. Darting time (T_{dart}), time from darting to the first signs of immobilization ($T_{1st\text{-}symptoms}$, e.g., tail dropping, slower pace, or Hackney gait), and time from darting to recumbency ($T_{recumbency}$) were recorded. Induction scores (Supplemental Figure 1) were assigned to evaluate drug combination effectiveness, as described by Pfitzer et al.[43] Scores of 1 or 2 indicated adequate immobilization, whereas scores 3 or 4 indicated inadequate immobilization and exclusion from the study.

After recumbency, the rhinoceroses were blindfolded and earplugs were inserted to minimize external stimuli. An 18-ga IV catheter (Jelco®, IV catheter radio-opaque; Smith Medical International, Upper Pemberton, Ashford TN25 4BF, United Kingdom) was placed in an auricular vein (allowing emergency treatment and naltrexone administration postprocedure), and the rhinoceroses were positioned in sternal recumbency for clinical and physiological data collection. Rectal temperature (RT) was measured by inserting a calibrated digital thermometer (Hanna Checktemp® 1, Hanna Instruments, Smithfield, RI 02917, USA) 10 cm into the rectum, ensuring the probe was in contact with the mucosa and not obstructed by feces. Peripheral arterial oxygen hemoglobin saturation (SpO_2) was assessed using a pulse oximeter (Nonin® PalmSat model 8500, Nonin Medical Inc., Plymouth, MN 55441, USA) with a transflectance probe placed under the third eyelid. RR was determined by visually observing chest movements or by feeling nasal airflow if chest movement was unclear. Blood pressure was continuously monitored via a 22-ga catheter (Introcan®-W, B. Braun, 34209 Melsungen, Germany) in a medial auricular artery, connected to a pressure transducer (Deltran II, Utah Medical, Midvale, UT 84047, USA) and an intra-arterial blood pressure monitor (IntraTorr, IntraVitals, Coventry TW11 8UB, United Kingdom). The catheter was flushed with heparinized saline before recording readings. HR was either recorded from the pulse rate measured by the IntraTorr monitor or by auscultation, with both methods showing close agreement when clinically compared. Variables recorded every 5 min included RT, SpO_2, HR, RR, and intra-arterial blood pressure. Arterial blood samples were obtained in preheparinized syringes from the intra-arterial catheter (Heparin Sodium Fresenius, 5,000 IU/1 ml, Fresenius Kabi (Pty) Ltd., Cape Town, 7475, South Africa) every 10 min after recumbency to measure blood gas variables, electrolytes, and metabolic biomarkers (glucose, lactate, and creatinine) by using an epoc® portable blood analyzer and epoc blood gas, electrolyte, and metabolite test cards (Epocal, Ottawa, Ontario K1G 3P5, Canada). Barometric pressures were obtained from the epoc analyzer's onboard barometer. None of the study rhinoceroses were given supplementary oxygen; therefore, the fraction of inspired oxygen was presumed to be 0.209 (20.9%).

Immobilization score (Supplemental Figure 2) was used to assess immobilization quality, ranging from 1 (conscious, requiring redosing) to 6 (excessively deep immobilization with absent reflexes and

cardiorespiratory distress), as described by Pfitzer et al.[43]

After completing dehorning, naltrexone was administered IV to antagonize the potent opioid's immobilizing effects and this time was recorded as $T_{reversal}$. Time to standing was recorded, and the recovery quality was subjectively scored (Supplemental Figure 3), with scores ranging from 1 (able to stand with one or two attempts) to 4 (prolonged recumbency requiring redosing), as described by Pfitzer et al.[43]

Statistical analysis

Statistical analysis was performed using RStudio version 3.6.1 (RStudio, PBC, Boston, MA 02210, USA). The physiological data collected over time were compared between treatment groups by using a linear mixed effects model, with fixed variables such as time, sex, RT, HR, RR, SpO_2, and blood pressure and the random variable of rhinoceros ID. Within-group comparisons, that is, changes over time, were made between all of the time points. Significant values ($P < 0.05$) were compared using a Bonferroni correction, allowing multiple pairwise comparisons to determine where the differences occurred. Differences between treatments/groups were analyzed both at each individual time point and across the averages for each treatment group. A Kruskal–Wallis test was used to determine differences in median induction, immobilization, and recovery scores between treatments. A one-way ANOVA was used to determine differences in time to initial effect, time to recumbency, time to first recovery, time to head up, time to standing, and time to walking between treatments. All data were analyzed for normality using a Shapiro–Wilk test. Hct, HR, mean arterial pressure, systolic pressure, and some of the epoc data (potassium, partial pressure of carbon dioxide in arterial blood [$PaCO_2$], and partial pressure of arterial oxygen [PaO_2]) were normally distributed, whereas epoc data (anion gap, base excess [BE], glucose, and lactate) were not and were log transformed. The remaining variables were not normally distributed even after transformation.[34]

RESULTS

All 24 rhinoceroses were successfully immobilized for dehorning by using the selected drug combinations, with no additional immobilizing drugs required and no morbidity or mortalities occurring. The mean $T_{1st\text{-}symptoms}$ was 1.7 ± 0.9 min in the EA group ($n = 11$, lost sight of one rhinoceros during induction) and 1.1 ± 0.4 min in the TA group ($n = 12$), with no difference between the groups ($P = 0.0719$). However, the $T_{recumbency}$ was faster in the TA group (2.9 ± 0.8 min) than in the EA group (3.9 ± 0.8 min) ($P = 0.0024$). Induction scores and quality of immobilization did not differ between groups ($P = 0.545$ and 0.3804, respectively), with all rhinoceroses having inductions scores of 1 or 2 and immobilization scores of 3 (light plane of immobilization) or 4 (moderate plane of immobilization).

RT for each treatment group is shown in Table 1. Although there was no significant difference between the groups ($P = 1.000$), RT increased over time in both groups ($P < 0.05$; Table 1). Similarly, there were no differences between treatment groups in SpO_2 ($P = 1.000$) or RR ($P = 1.000$), and no significant changes were observed over time within either group.

HR did not differ between treatment groups ($P = 0.1722$); however, it decreased from 15 to 30 min compared with 5 min in both groups ($P < 0.05$). Throughout the entire immobilization hypertension was present in the EA treatment group as well as in the TA treatment group. Blood pressure (systolic, mean, and diastolic) decreased over time in both groups, with significantly lower values recorded after 25 and 30 min of recumbency ($P < 0.05$; Table 1; Fig. 1), but no differences were observed between groups.

In total, 70 blood gas samples were collected (36 in the EA group and 34 in the TA group). The average PaO_2 was 64.0 ± 3.6 mmHg in the EA group and 58.0 ± 2.6 mmHg in the TA group, with no significant differences between groups ($P = 0.922$) or over time ($P = 0.6501$). Similarly, $PaCO_2$ did not differ between groups or time points ($P = 1.000$). The pH increased significantly over time in both groups ($P < 0.001$; Fig. 2), and BE increased from 10 minutes after recumbency (T_{10}) to T_{30} ($P < 0.0002$) in both groups, with no differences between groups ($P = 1.000$). Lactate levels also decreased significantly over time ($P < 0.001$; Fig. 3) in both groups, without differences between groups ($P = 1.000$). There were no significant differences between groups or over time for bicarbonate (HCO_3^-), glucose, Hct, creatinine, anion gap, or electrolytes ($P > 0.05$).

Recovery was monitored in 11 rhinoceroses from the EA group and 10 from the TA group. There was no difference in recovery score between groups ($P = 0.682$), with all rhinoceroses receiving a score of 1, indicating swift recovery. The time from naltrexone injection to standing was 1.6 ± 1.1 min in the EA group (range, 0.3–3.8 min) and 1.5 ± 0.2 min in the TA group (range, 1.3–1.9 min). All rhinoceroses recovered fully from the immobilization.

Table 1. Mean physiological variables for each treatment group over time for 24 black rhinoceroses (*Diceros bicornis*), immobilized with either EA or TA. When there were significant changes over time (i.e., the treatment-by-time interaction was significant, $P < 0.05$), values are in bold, with an asterisk (*) showing data that differed from initial value (T_5 for clinical parameters or T_{10} for the blood gas evaluation) within the same treatment group. There were no significant differences between treatments.[a]

		Time after recumbency (min)						
	Treatment	5	10	15	20	25	30	Mean ± SD (min – max)
HR (bpm)	EA	**79**	77	**77***	**74***	**73***	**70***	75 ± 8 (56–91)
	TA	**91**	92	**88***	**85***	**82***	**80***	86 ± 10 (63–115)
RT (°C)	EA	**37.9**	37.8	**37.9***	**37.9***	**38.0***	**38.0***	37.9 ± 0.8 (36.4 – 39.0)
	TA	**37.3**	37.7	**37.8***	**37.9***	**37.8***	**37.9***	37.7 ± 0.6 (35.8 – 38.7)
RR (bpm)	EA	8	7	6	6	6	6	7 ± 2 (4–12)
	TA	6	7	6	6	6	7	6 ± 2 (3–10)
SpO_2 (%)	EA	94	92	92	93	93	95	93 ± 5 (80–100)
	TA	95	93	93	95	96	96	95 ± 4 (85–100)
SAP (mmHg)	EA	**210**	193	191	181	**175***	**174***	184 ± 22 (133 – 235)
	TA	**206**	199	194	189	**182***	**177***	188 ± 25 (132 – 234)
MAP (mmHg)	EA	**186**	177	172	168	**162***	**161***	169 ± 15 (126 – 198)
	TA	**182**	177	174	170	**166***	**159***	169 ± 20 (126 – 213)
DAP (mmHg)	EA	**167**	162	159	157	**150***	**150***	157 ± 14 (121 – 187)
	TA	**166**	162	163	156	**153***	**145***	156 ± 18 (126 – 208)
PaO_2 (mmHg)	EA		61.1		62.8		68.1	64.0 ± 3.6 (42.8 – 98.8)
	TA		55.1		59.3		59.9	58.0 ± 2.6 (37.2 – 77.8)
$PaCO_2$ (mmHg)	EA		54.4		50.9		48.2	51.2 ± 3.1 (35.2 – 66.0)
	TA		55.4		53.4		54.7	54.5 ± 1.0 (40.1 – 66.0)
A-a gradient (mmHg)	EA		32.0		34.3		31.4	32.6 ± 7.9 (9.4 – 48.4)
	TA		38.3		36.5		37.1	37.3 ± 12.2 (7.5 – 57.4)
pH	EA		**7.23**		**7.25***		**7.29***	7.25 ± 0.03 (7.11 – 7.39)
	TA		**7.22**		**7.25***		**7.25***	7.25 ± 0.02 (7.10 – 7.33)
HCO_3^- (mmol L^{-1})	EA		22.3		22.5		23.2	22.7 ± 0.45 (16.9 – 27.9)
	TA		22.7		23.7		24.0	23.47 ± 0.68 (16.2 – 29.6)
BE (mmol L^{-1})	EA		**−5.4**		**−4.7***		**−3.4***	−4.51 ± 0.99 (−12.2 – 1.6)
	TA		**−5.1**		**−3.5***		**−3.3***	−3.95 ± 0.99 (−13.3 – 2.6)
Agap (mmol L^{-1})	EA		12		12		11	11.35 ± 0.52 (7 – 12)
	TA		14		12		13	12.74 ± 1.04 (6 – 20)
Lactate (mmol L^{-1})	EA		**6.61**		**4.56***		**3.58***	4.92 ± 1.55 (1 – 12.1)
	TA		**6.64**		**4.48***		**3.59***	4.90 ± 1.57 (0.9 – 15.0)

[a] EA, etorphine, azaperone, and hyaluronidase; TA, thiafentanil, azaperone, and hyaluronidase; RT, rectal temperature; SAP, systolic arterial pressure; MAP, mean arterial pressure; DAP, diastolic arterial pressure; PaO_2, partial pressure of arterial oxygen; $PaCO_2$, partial pressure of carbon dioxide in arterial blood; A-a, alveolar-arterial; HCO_3^-, bicarbonate; BE, base excess; Agap, anion gap.

DISCUSSION

This study evaluated the physiological effects and clinical outcomes of two immobilization protocols—EA and TA—in black rhinoceros to assess their relative safety and efficacy. Both protocols were effective in inducing and maintaining chemical immobilization for at least 40 min without the need for additional anesthetic agents. Their reliability and reversibility minimized the risks of repeated darting, supplementary drug administration, and prolonged restraint and ensured safe recovery, thereby supporting both animal welfare and personnel safety. No mortalities or morbidities were observed. In addition, no significant differences were observed between groups for most physiological variables, highlighting their comparable impacts on the rhinoceroses. These findings expand the limited knowledge on thiafentanil use in black rhinoceros and underscore the need for species-specific evaluations to guide the selection of immobilization protocols, especially in endangered species such as the black rhinoceros.

Comparison of the two treatment groups showed no differences in the time from darting to the first signs of induction. However, rhinoceroses darted with the TA combination became recumbent 1 min 4 s faster than those immobilized with the EA combination. Induction quality was excellent or good in all cases. Once recumbent, there was sufficient relaxation to allow manipulation of the rhinoceros and no additional immobilization drugs were necessary to improve the immobilization. This difference in time to recumbency, although seemingly minor, is of significant practical importance in black rhinoceros. These animals are known for

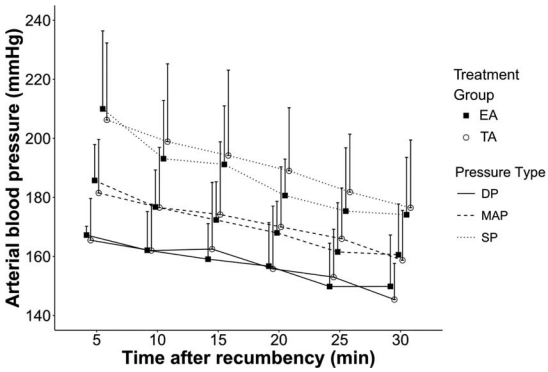

Figure 1. Arterial blood pressure (mean ± SD) of each treatment over time for 24 black rhinoceros (*Diceros bicornis*), immobilized with either etorphine, azaperone, and hyaluronidase or thiafentanil, azaperone, and hyaluronidase. Note that for clarity, the values of each drug combination at specific time points are slightly offset.

their unpredictable and often hazardous response to helicopter darting, which can include running into dense bush or topographical hazards such as rivers or ravines. A faster immobilization greatly reduces the risk of the rhinoceros injuring itself

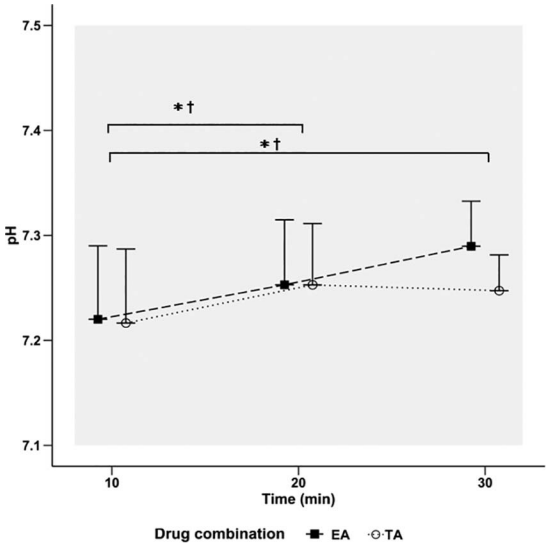

Figure 2. The pH (mean ± SD) in free-ranging black rhinoceros (*Diceros bicornis*) immobilized with either etorphine, azaperone, and hyaluronidase (EA) or thiafentanil, azaperone, and hyaluronidase (TA). Note that for clarity, the values of each drug combination at specific time points are slightly offset; shaded area represents values of the pH, as reported in black rhinoceros[51]; $*P < 0.05$ EA T_{10} vs $T_{20–30}$ and $\dagger P < 0.05$ TA T_{10} vs $T_{20–30}$, no difference between treatment groups ($P > 0.05$, linear mixed effects model).

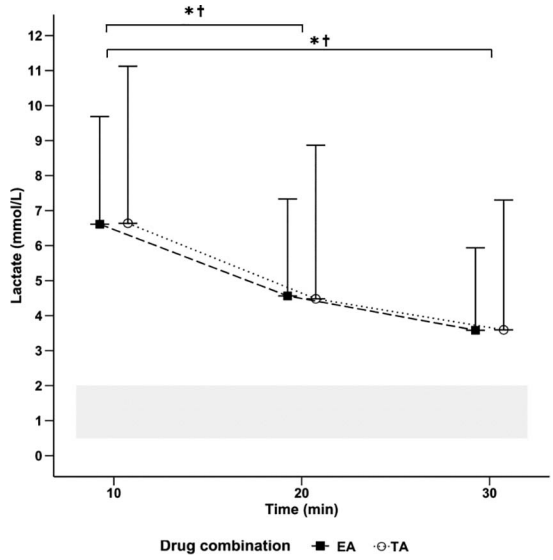

Figure 3. Lactate (mean ± SD) in free-ranging black rhinoceros (*Diceros bicornis*) immobilized with either etorphine, azaperone, and hyaluronidase (EA) or thiafentanil, azaperone, and hyaluronidase (TA). Note that for clarity, the values of each drug combination at specific time points are slightly offset; shaded area represents values of the lactate, as reported in healthy horses[14]; $*P < 0.05$ EA T_{10} vs $T_{20–30}$ and $\dagger P < 0.05$ TA T_{10} vs $T_{20–30}$, no difference between treatment groups ($P > 0.05$, linear mixed effects model).

during this vulnerable period. By reducing induction time by more than a minute, the TA combination provides a clear advantage in minimizing the risk of serious physical trauma during movement through potentially dangerous terrain.

Rapid recumbency (induction) times during wildlife immobilizations are critical to limit capture-related morbidities, including capture-induced hyperthermia. Because the primary cause of capture-induced hyperthermia is the physiological stress response to pursuit and restraint,[33] limiting the duration of exposure to these stressors before immobilization is important to mitigate the rise in the body temperature. Furthermore, immobilization drugs can impair thermoregulation, leading to a state of poikilothermia.[26] Consequently, heightened stress responses, metabolic activity, and/or elevated environmental temperatures can result in an increase in body temperature. Hyperthermia can result in cellular damage, but it also indicates that other capture-induced metabolic derangements have occurred in an animal,[26,50] with both possibly causing secondary complications such as

myopathy.[4,40] In this study, RTs ranged from 35.8 to 39°C, aligning with reference values for black rhinoceros (36–39°C) reported by Morkel.[38] Although RTs increased during immobilization, this may have been influenced by ambient conditions, which unfortunately were not measured. High ambient temperatures, typical of the study location, could exacerbate hyperthermia during immobilization due to the animal's drug-induced thermoregulatory dysfunction, although initial capture-induced hyperthermia primarily reflects the stress response to capture, independent of ambient temperature.[4,31] It must be noted that, in large animals such as rhinoceroses and elephants, their considerable body mass confers thermal inertia (i.e., the capacity to buffer rapid changes in core body temperature by temporarily storing excess heat). This means that ambient conditions have a slower and less immediate impact on the body temperature.[13] This effect is possibly evident in the etorphine treatment group, where body temperature increased gradually throughout immobilization. By contrast, the thiafentanil group showed a more rapid rise in body temperature during the first 10 min, suggesting potential drug-specific differences.

Although raw mean temperatures appeared similar at some time points, the model detected significant differences. This is because estimated marginal means (EMM), which adjust for repeated measures and individual variation, revealed consistent trends in temperature over time. EMM provide a more accurate comparison by accounting for the structure of the data and controlling for confounding effects.

In this study, black rhinoceroses immobilized with EA or with TA experienced deep and regular RR within the reference range reported for immobilized black rhinoceroses (4–8 bpm), although below-normal levels for conscious rhinoceroses (unsedated black rhinoceroses: 8–12 bpm).[7,35] The RR remained stable in both groups, but one rhinoceros in the TA group reached a low of 3 bpm at T_{20}. SpO_2 levels were consistently below 95% in both groups, but improved to slightly above 95% in the TA group after T_{20}. Despite these findings, both groups exhibited moderate-to-severe hypoxemia,[7,18] with mean PaO_2 values of 64 and 58 mmHg in the EA and TA group, respectively, and no differences between them. Severe clinically relevant hypoxemia in humans is defined as a $PaO_2 < 60$ mmHg and a $SpO_2 < 90\%$.[19]

Hypoxemia was likely due to opioid-induced respiratory depression, resulting in hypoventilation and impaired CO_2 elimination (indicated by the mildly elevated $PaCO_2$ seen in both groups). In addition, diffusion impairment at the alveolar membrane, indicated by elevated alveolar-arterial gradients (32.6 ± 7.9 mmHg in the EA group and 37.3 ± 12.2 mmHg in the TA group), suggested poor oxygen transfer between alveoli and capillaries. These values exceeded normal ranges reported for horses, a close relative of the rhinoceros.[16] Previous studies have shown that potent opioids can induce similar respiratory compromise in other species, including goats, impala (*Aepyceros melampus*), and white rhinoceros.[2,15,29] These effects are induced mostly via the activation of μ-opioid receptors, and respiratory depression is reported to be more prominent in white rhinoceros than black rhinoceros.[1,19,52] However, the current results indicate that black rhinoceroses are equally susceptible to opioid-induced respiratory depression and it might be beneficial for these animals to also receive butorphanol (IV). Agents such as butorphanol, in combination with oxygen insufflation, can be used to partially alleviate severe respiratory depression and hypoxemia in animals immobilized with opioids by partially antagonizing the μ-receptors, as shown in white rhinoceros.[5,32] Butorphanol administration in black rhinoceroses immobilized with etorphine or thiafentanil requires caution because these animals are highly sensitive to this partial agonist-antagonist, and it might cause sudden arousal.[32]

Both treatment groups exhibited tachycardia compared with standing, unrestrained white rhinoceroses.[7] Elevated HR likely contributed to increased blood pressures in all rhinoceroses, alongside potential increases in stroke volume and systemic vascular resistance, although these are difficult to assess in the field. All rhinoceroses were hypertensive compared with standing white rhinoceroses.[7] Blood pressures (systolic arterial pressure, mean arterial pressure, and diastolic arterial pressure) declined over time, reaching significantly lower levels after 25–30 min, but they remained elevated in both groups, with no differences between treatments. Prolonged hypertension is shown to cause pulmonary hypertension in goats,[29] may increase cardiovascular stress, alter tissue and organ perfusion, or contribute to secondary complications such as myopathy.[30]

Azaperone is crucial in rhinoceros immobilization not only for its tranquilizing effects but also due to its peripheral α-1–mediated vasodilatory effects, counteracting opioid-induced hypertension.[19,25] However, excessive azaperone can cause severe hypotension and respiratory impairment, as seen in blesbok (*Damaliscus pygargus phillipsi*),[15] and

more recently, white rhinoceroses.[5] Despite adequate dosages for immobilization, the high blood pressures observed in this study suggest a potential benefit in increasing the azaperone dose. The azaperone dose used was lower than previously reported for black rhinoceros.[37,38,46] Historically, doses up to 250 mg were used[37] but their physiological effects remain underreported.

Respiratory depression, indicated by increased $PaCO_2$, likely led to respiratory acidosis and the acidemia observed, although pH and HCO_3^- levels improved slightly over time. Elevated lactate levels were seen initially, possibly because of hypoxemia, exertion, and the stress induced by capture, but they decreased as immobilization progressed, suggesting improved tissue oxygenation and a waning stress response over time.[49] The rise in anion gap and lactate, along with decreased HCO_3^- and negative BE, confirmed the presence of metabolic lactic acidosis. Therefore, the rhinoceroses in both treatment groups had a mixed respiratory and metabolic acidosis. These results align with findings of acidemia, hypoxemia, and hypercapnia in white[5,8] and black[12,27,41] rhinoceroses immobilized with etorphine and azaperone. Electrolytes remained stable over the immobilization period, and there were no differences between treatment groups in these or the other physiological variables.

Although a lower ratio of naltrexone to opioid was used in the rhinoceroses in the TA group, recovery from the immobilization was smooth and uneventful in all rhinoceroses after the administration of the opioid antagonist naltrexone IV. All rhinoceroses were mobile within 3 min 50 s, and no differences between the treatment groups were detected. Postimmobilization monitoring was performed by the antipoaching units. Renarcotization was not noticed in any instances, confirming the efficacy of the pure, long-acting antagonist naltrexone at the suggested doses.

The study faced several limitations, including challenges in determining the exact body weight of rhinoceroses, leading to potential minor under- or overdosing due to reliance on visual estimates. Aging rhinoceroses from the helicopter was challenging at times, especially without the presence of a calf for reference. Practical and ethical constraints prevented the inclusion of a control group or a double crossover design, whereas arterial blood sampling was often difficult due to catheter issues, requiring alternative arterial collection methods. Recovery data were limited, with some observations recorded via GoPro (San Mateo, CA 94402, USA), due to the fast, often aggressive recovery of rhinoceroses. The epoc blood analysis system, although commonly used, has not been validated for use in wildlife or black rhinoceros specifically.

Future research on the efficacy of oxygen insufflation, fluid, and/or butorphanol administration is recommended to determine whether these interventions could further enhance immobilization safety in black rhinoceros.

CONCLUSION

The EA and TA combinations both effectively immobilized free-ranging black rhinoceros, with no significant differences observed in their effects on physiological parameters, immobilization quality, or recovery. However, the thiafentanil combination resulted in a notably faster induction time, offering a clear advantage in situations where rapid recumbency is critical to minimize risk during field immobilizations. The observed hypoxemia in both groups underscores the need for continued monitoring of respiratory variables during immobilization. Future studies should focus on refining opioid protocols to mitigate respiratory compromise while ensuring effective immobilization. These findings contribute valuable insights for the conservation management of this critically endangered species and also emphasize the importance of optimizing drug protocols to reduce immobilization-related risks.

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Supplemental Figure 1. Qualitative scoring of the induction, based on signs observed following darting (induction score), as described by Pfitzer et al.[43]

Supplemental Figure 2. Qualitative scoring of the immobilization, based on the signs observed following initial recumbency (immobilization score), as described by Pfitzer et al.[43]

Supplemental Figure 3. Assessment of the efficacy of naltrexone's antagonistic effects following IV administration through qualitative scoring, based on the observed clinical signs (recovery score) and their recommended action, as described by Pfitzer et al.[43]