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ORIGINAL RESEARCH

Efficacy and safety of three different opioid-based immobilisation combinations in blesbok (*Damaliscus pygargus phillipsi*)

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African wildlife species are increasingly being immobilised with combinations of a low dose of potent opioids combined with medetomidine and azaperone. The physiological effects of these combinations in comparison to conventional potent opioid-azaperone combinations have scarcely been evaluated. In this cross-over study conducted on eight captive blesbok, we compared the physiological variables of blesbok immobilised with 2 mg of thiafentanil + 10 mg of azaperone (TA); 0.5 mg thiafentanil + 1.5 mg medetomidine (TM), and 0.5 mg thiafentanil + 1.5. mg medetomidine + 10 mg azaperone (TMA). Thiafentanil's effects were antagonised with naltrexone at 10 mg naltrexone per mg thiafentanil, and the medetomidine effects with atipamezole at 5 mg atipamezole per mg medetomidine. The physiological variables were compared between treatment groups using descriptive statistics and repeated measures ANOVA. The TA combination resulted in the shortest induction and recovery times, higher heart rates, respiratory rates, PaO_2, SpO_2, and lower MAP and A-a gradients, but with less muscle relaxation. The TM and TMA combinations caused marked bradycardia and hypoxaemia. The hypoxaemia was most severe in animals immobilised with TMA, and four of eight blesbok immobilised had a $PaO_2 < 35$ mmHg at the 10- or 15-minute sampling point. These blesbok were provided supplementary oxygen, which corrected the hypoxaemia. The TA combinations caused the lowest degree of physiological compromise. All three combinations were effective for the immobilisation of blesbok, but as the low-dose thiafentanil and high-dose medetomidine combinations caused marked hypoxaemia, supplementary oxygen is recommended when using these combinations.

Keywords: thiafentanil, medetomidine, azaperone, chemical immobilisation, hypoxaemia

Introduction

Thousands of wild animals are being immobilised each year for various purposes, including collaring, translocation or veterinary treatment. The drug combination used depends on the species immobilised, but for free-ranging African bovids and antelope species, a combination a potent opioid, such as etorphine or thiafentanil, plus a butyrophenone, such has azaperone, and, or, an $alpha_2$ adrenergic agonist, such as medetomidine, is frequently used (Kock & Burroughs 2021). In recent years, mixtures containing a very low dose of potent opioid combined with a higher dose of the $alpha_2$ adrenergic agonist medetomidine, either alone or in combination with azaperone, have gained increasing popularity (Kock & Burroughs 2021). The shift towards using a low dose of potent opioids combined with a higher dose of a sedative is partially motivated by the high cost and limited availability of potent opioids.

The potent opioid thiafentanil is a fentanyl analogue with exclusive affinity for μ-opioid receptors (Vardanyan & Hruby 2014). Similarly to etorphine it causes a fast induction, analgesia, hypertension, muscle excitation, and dose-dependent respiratory depression (Kukanich & Papich 2013; Gaudio et al. 2020; Pfitzer et al. 2021). Because of the side-effects, thiafentanil is often combined with sedatives or tranquilisers.

Azaperone, a tranquiliser of the butyrophenone family, is often used for short-term tranquilisation or in combination with opioids and/or $alpha_2$ adrenergic agonists (Szabó et al. 2015; Semjonov et al. 2018; Gaudio et al. 2020). Butyrophenones have dopaminergic (D_2) antagonistic effects, resulting in tranquilisation and the potentiation of immobilisation (Gaudio et al. 2020; Gaudio et al. 2021; Laubscher et al. 2022). Further, azaperones' effect on the $alpha_1$-adrenergic receptors results in peripheral vasodilation (Lees & Serrano 1976). In some species, such as African elephants that are sensitive to opioid-induced hypertension, the addition of azaperone may reduce the risk of pulmonary oedema (Hattingh et al. 1994; Still et al. 1996; Buss et al. 2022; Chelopo et al. 2022). Although it is largely reported that butyrophenones have a minimal effect on respiration, this may be species-specific with recent research indicating that it may cause cardiorespiratory compromise in wild ungulates when used in combination with potent opioids (Pfitzer 2019; Gaudio et al. 2020; Gaudio et al. 2021).

$Alpha_2$ adrenergic agonists such as medetomidine, produce sedation, analgesia and muscle relaxation by stimulating $alpha_2$ adrenergic receptors, pre- and post-synaptically in tissues throughout the body (Jalanka & Roeken 1990; Fahlman 2008). Cardiovascular effects of $alpha_2$ adrenergic agonists include an initial increase in blood pressure and reflex bradycardia due to peripheral vasoconstriction followed by centrally mediated reduction in sympathetic tone, heart rate, blood pressure and cardia output (Posner 2018). The extent and duration of this effect, however, appears to be dose- and species-dependent

with some reports indicating no hypertension or persistent hypertension throughout the total period of immobilisation (Kalema-Zikusoka et al. 2003; Murrell & Hellebrekers 2005; West et al. 2007). The respiratory effects of alpha$_2$ adrenergic agonists include centrally-mediated depression of respiratory rates and reduction in minute ventilation (Posner 2018). In sheep, the administration of alpha$_2$ adrenergic agonists results in activation of pulmonary intravascular macrophages leading to endothelial and alveolar damage and subsequent oedema (Doherty et al. 1986; Celly et al. 1997; Ranheim et al. 2008). In a number of wild ungulates hypoxaemia is a significant problem when alpha$_2$ adrenergic agonists are included in the immobilising drug combinations (Read 2003; Risling et al. 2011; Lian et al. 2017).

To date, little published information is available on the low-dose opioid/ high dose alpha$_2$ adrenergic agonist combinations. Furthermore, there is no data on whether the addition of azaperone improves or reduces the safety of these combinations. Anecdotal reports on the effects of these combinations on the respiratory and cardiovascular system are also mixed, which is unsurprising considering the variable effects of opioids, alpha$_2$ adrenergic agonists and butyrophenones on these systems. The aim of this study was therefore to investigate and compare the immobilising and physiological effects of the following three combinations in blesbok (*Damaliscus pygargus phillipsi*): 1.) recommended-dose thiafentanil + recommended-dose azaperone; 2.) low-dose thiafentanil + high-dose medetomidine; 3.) low-dose thiafentanil + high-dose medetomidine + recommended-dose azaperone. Blesbok were selected as the research species since they are abundant in the study area, adapt well to captivity and have previously been used in other research projects as a representative species for African ungulates (du Plessis 2018; Semjonov et al. 2018; Gaudio et al. 2020; Pfitzer et al. 2020; Gaudio et al. 2021; Pfitzer et al. 2021; Laubscher et al. 2022).

Research methods and design

Animals and housing

The study was carried out at Wildlife Pharmaceutical's Wildlife Research Facility (25°31'25.2" S, 31°06'50.8" E). Eight wild-caught adult female blesbok (56.8 ± 4.9 kg) were translocated and acclimatised at the research facility, in boma enclosures (6 x 8 m), for four weeks before the start of the study. On arrival, all the animals underwent a health check which included a physical exam, blood tests, faecal egg counts and blood smears for differential cell counts. Each animal was weighed, and their horns were piped and marked with white tape for identification. They were provided feed and water *ad libitum* and allowed to move between enclosures in between trials.

Study design

The study was conducted as a cross-over study in which all blesbok were immobilised three times with a two-week washout period between treatments. A sample size of eight animals was the minimum to allow for the detection of differences in respiratory rates, as low as four breaths per minute, with 95% confidence and 80% power. Treatments were allocated randomly. All the animals received each of the following treatments intramuscularly (IM):

1.) TA: 2 mg thiafentanil (Thianil, 10 mg/ml, Wildlife Pharmaceuticals [Pty] Ltd., Mpumalanga, South Africa) + 10 mg azaperone (Stresnil, 40 mg/ml, Elanco Animal Health [Pty] Ltd., Gauteng, South Africa). Reversal with 20 mg naltrexone (Trexonil, 50 mg/ml, Wildlife Pharmaceuticals [Pty] Ltd., Mpumalanga, South Africa);

2.) TM: 0.5 mg thiafentanil + 1.5 mg medetomidine (10 mg/ml, Kyron Laboratories [Pty] Ltd., Gauteng, South Africa). Reversal with 5 mg naltrexone and 7.5 mg atipamezole (Antisedan, 5 mg/ml, Zoetis South Africa [Pty] Ltd., Gauteng, South Africa); and

3.) TMA: 0.5 mg thiafentanil + 1.5 mg medetomidine + 10 mg azaperone.

Reversal with 5 mg naltrexone and 7.5 mg atipamezole.

Immobilisation and monitoring

The animals were immobilised from a distance of 10–15 m *via* a CO_2 powered dart rifle (DanInject, 6000 Kolding, Denmark) using a 1.5 ml plastic dart with a 25 mm collared needle (DanInject). All darting was done by the same person and the dart site was the gluteal muscles in all animals. Time of injection to when the animal showed first signs of altered consciousness (time to first sign) and to when the animal became recumbent (induction time) were recorded. Once recumbent, the animal was blindfolded, placed on a stretcher, and moved from the enclosure to a nearby shaded area for monitoring. The blesbok was maintained in sternal recumbency on a table and its horns were held by a handler so that the head was elevated above the thorax, with the neck straight and the nose pointing downwards. A 22-gauge Jelco® catheter (Midlands Veterinary Wholesalers, Gauteng, South Africa) was inserted into either the auricular artery or the medial artery of the metacarpus. Physiological measurements – heart rate (HR), respiratory rate (RR), mean arterial blood pressure (MAP), peripheral haemoglobin oxygen saturation (SpO_2), and rectal temperature (RT) – were recorded every 5 min of monitoring, starting at 7 min after recumbency, and lasting for 35 min. Subjective assessment of the quality of immobilisation was done as described by Laubscher et al. (2022). Heart rate and MAP were recorded using a portable monitor (IntraTorr, IntraVitals, United Kingdom) connected to the arterial catheter. SpO_2 was assessed by means of a pulse oximeter (Nonin PalmSat 2500, Tilburg, Netherlands) with a reflectance probe fixed with tape to the skin under the tail. The rectal temperature was measured with a digital thermometer (Hanna Checktemp 1, Hanna Instruments (Pty) Ltd., NE, USA). The respiratory rate was measured by visual assessment of inhalations and exhalations.

Arterial blood samples were collected from the catheter at 10, 15, 25 and 35 min after recumbency. Blood gas analysis was performed using a portable analyser (EPOC Reader Blood Analysis and pre-calibrated EPOC BGEM smart cards, Epocal, Kyron Laboratories, Gauteng, South Africa). Variables measured included partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), and lactate (Lact). All variables were measured at 37 °C. The EPOC reader also recorded barometric pressure at the time of sampling as well as environmental temperature. A $PaO_2 < 35$ mmHg was set as a cutoff for immediate oxygen administration.

At the end of monitoring, the dart wound was treated, and the animal weighed. The animal was then carried back to its enclosure, removed from the stretcher, placed in sternal recumbency, and the antagonist/s administered IM to reverse the immobilisation. The time from injection of the antagonists until the animal stood up (recovery time) was recorded.

Statistical analysis

Data was summarised using descriptive statistics (Tables I and II) and the mean and standard deviation of each variable graphed by drug combination for each time point (Figures 1 and 2). The alveolar-arterial oxygen partial pressure gradient $P(A\text{-}a)O_2$ was calculated as PAO_2 - PaO_2, and PAO_2 determined as previously described (Meyer et al. 2015). Overall significant differences in physiological variables between the three treatment groups was assessed using repeated measures Analysis of Variance with the R package rstatix (Kassambara, 2021). Differences between physiological variables at each time point was assessed using the Bonferroni post hoc test for multiple comparisons. Graphs were created using the R package ggplot2 (Bates et al. 2015; Wickham 2016). *P*-values of ≤ 0.05 were considered significant. Summary statistics was conducted using STATA (Stata I/C 15.1, StataCorp, College Station, Texas, USA).

Ethical considerations

All procedures performed in this study followed international, national, and/or institutional guidelines for the care and use of animals. The study was approved by both Wildlife Pharmaceutical's Animals Ethics Committee (Approval number: WPAEC-2022-VATINOXAN-51-B) and the University of Pretoria's Animal Ethics Committee (Approval number: REC083-21).

Results

The mean (standard deviation) dose of TA was 0.04 (0.003) mg/kg thiafentanil and 0.18 (0.02) mg/kg azaperone. For TM, the dose administered was 0.01 (0.001) mg/kg thiafentanil, 0.03 (0.003) mg/kg medetomidine, with the same doses of thiafentanil and medetomidine, and the addition of 0.18 (0.02) mg/kg azaperone, for TMA.

The mean induction time (time from darting to recumbency) when the blesbok were immobilised with TA was 3:50 min (SD = 1:12 min, range = 2:00–3:57 min). When the blesbok were immobilised with TM, the induction time averaged 14:34 min (SD = 6:46 min, range 6:00–27:21 min), whereas when TMA was used it was 8:13 min (SD = 2:47 min, range = 5:00–14:08 min). Two blesbok, when immobilised with TM, did not become recumbent within 30 minutes and were re-immobilised with the same combination several weeks later, at which time the immobilisation was successful. The data from the second immobilisation is included in the analysis. After recumbency, the animals immobilised with the TM and TMA combinations had a high degree of muscle relaxation and complete lack of movement, whereas the animals immobilised with TA exhibited chewing, ear, and tail movements despite being fully immobilised.

Recovery time (from administration of the reversal intramuscularly to standing) averaged 2:19 min (SD = 0:37 min, range 1:40–3:38 min) for the blesbok when immobilised with TA; 7:29 min (SD = 2:56, range 3:10–13:30 min) when immobilised with TM and 10:31 min (SD = 3:39 min, range 4:30–10:48 min) when immobilised with the TMA combination.

Heart rate was significantly higher in blesbok immobilised with TA compared to both TM and TMA at all time points (all $p < 0.001$) and there was no significant difference in heart rates between blesbok immobilised with TM or TMA (Table I, Figure 1). Respiration rates were significantly higher in blesbok immobilised with TA compared to TM at the 15 ($p = 0.01$) and 30 minute ($p = 0.05$) time points and significantly higher in blesbok immobilised with TM compared to TMA at the 15 minute time point only ($p = 0.03$) Table I, Figure 1).

The oxygen saturation was significantly higher in the blesbok immobilised with TA compared to TMA at the 35 minute time point ($p = 0.04$) (Table I, Figure 2). Mean arterial pressures were significantly lower with the TA combination compared to TM at the 20, 25, 30, and 35 minute time points (all $p < 0.03$) and to TMA at the 15, 20, 25, 30, and 35 minute time points (all $p < 0.01$) (Table I, Figure 1).

In four of the blesbok immobilised with TMA, the PaO_2 was < 35 mmHg at the first or second sampling (10 or 15 minutes), and these animals subsequently received continuous intranasal oxygen treatment at a flow rate of 2 L/min, which corrected the severe hypoxaemia. Subsequent data from these animals were removed before analysis as it was expected that oxygen administration would influence the physiological values collected and no longer make them comparable to the rest of the dataset. All blesbok became hypoxaemic, but the PaO_2 (mmHg) in arterial blood was significantly higher with TA compared to TM at the 15, 25, and 35 minute time points ($p < 0.01$), and compared to TMA at all time points (all $p < 0.001$) (Table II, Figure 2). The PaO_2 was also significantly higher in blesbok immobilised with TM compared to TMA at the 25 and 35 minute time points ($p < 0.05$) (Table II, Figure 2). Overall, the $PaCO_2$ significantly increased over time ($p < 0.001$) and the $PaCO_2$ was significantly higher in blesbok immobilised with TMA compared to TM at the 10 and 25 minute time points ($p = 0.02$) and to TA at the 25 minute time point ($p = 0.01$) (Table II, Figure 2). The A-a gradient was significantly higher with TMA and TM compared to TA at all time points (all $p < 0.02$), and overall, the A-a gradient decreased over time ($p < 0.01$) (Table II, Figure 2). There were no significant differences in rectal temperature (Table I) or lactate (Table II) between treatments.

Discussion

All three combinations resulted in immobilisation for the duration of the observation period, however, induction times were significantly longer with the two combinations containing medetomidine. A short induction time, or at a minimum a short flight distance after darting, is desirable when immobilising free ranging animals in the field to reduce the risk of capture myopathy or losing track of the animal (Kock & Burroughs 2021). When immobilising animals in a boma, a longer induction time is less problematic, provided that the animal stays calm during the induction phase. Two blesbok did not become immobilised

Table I: Mean physiological variables ± SD (range) recorded during immobilisation of blesbok with TA (thiafentanil + azaperone), TM (thiafentanil + medetomidine) and TMA (thiafentanil + medetomidine + azaperone)

Time point (min)	Treatment	HR (bpm)	RR (breaths/min)	RT (°C)	SpO_2 (%)	MAP (mmHg)
7	TA	50.1 ± 10.8 (40–72)[a,b]	20.5 ± 8.7 (12–40)	38.3 ± 0.8 (37.0–39.4)	94.3 ± 5.1 (86–100)	111.3 ± 14.6 (91–134)
	TM	33.0 ± 4.5 (28–40)[a]	17.9 ± 8.8 (8–30)	38.9 ± 0.7 (37.9–39.8)	88.5 ± 12.2 (67–98)	114.0 ± 10.2 (98–125)
	TMA	34.0 ± 4.3 (28–40)[b]	14.5 ± 2.9 (12–20)	38.6 ± 0.5 (38.1–39.2)	90.5 ± 8.5 (75–100)	116.6 ± 5.6 (109–124)
10	TA	51.0 ± 10.2 (40–72) [a,b]	20.5 ± 5.9 (12–32)	38.5 ± 0.5 (37.6–39.3)	96.6 ± 2.3 (92–99)	109.6 ± 16.7 (89–134)
	TM	33.8 ± 4.8 (24–40)[a]	15.1 ± 10.3 (7–30)	38.9 ± 0.6 (37.9–39.8)	91.1 ± 10.1 (71–98)	112.0 ± 10.0 (100–125)
	TMA	34.6 ± 4.2 (28–40) [b]	13.0 ± 3.5 (8–16)	38.5 ± 0.8 (37.0–39.3)	89.0 ± 7.6 (75–100)	118.1 ± 7.6 (109–127)
15	TA	50.8 ± 8.2 (44–68) [a,b]	22.0 ± 8.2 (12–40)[a,b]	38.4 ± 0.5 (37.6–39.2)	95.9 ± 3.3 (91–100)	102.6 ± 14.1 (86–123)[b]
	TM	32.0 ± 3.6 (28–36) [a]	12.4 ± 4.8 (5–20)[a]	38.7 ± 0.7 (37.8–40.0)	91.3 ± 10.3 (68–100)	113.1 ± 8.2 (101–123)
	TMA	33.0 ± 2.8 (28–36)[b]	13.3 ± 4.4 (8–20)[b]	38.6 ± 0.6 (37.8–39.4)	86.8 ± 9.3 (72–990)	121.0 ± 7.3 (109–130)[b]
20	TA	49.0 ± 7.6 (40–64) [a,b]	19.3 ± 8.4 (8–36)	38.3 ± 0.6 (37.1–38.9)	95.8 ± 3.7 (90–100)	94.9 ± 14.1 (78–118)[a,b]
	TM	32.5 ± 3.8 (28–36)[a]	11.3 ± 5.6 (4–20)	38.8 ± 0.8 (37.6–40.0)	92.4 ± 6.1 (83–100)	112.9 ± 8.7 (101–123)[a]
	TMA*	36.0 ± 0.0 (36–36 [b]	13.5 ± 4.4 (8–18)	38.9 ± 0.6 (38.2–39.4)	88.5 ± 9.3 (77–99)	123.5 ± 8.7 (113–132)[b]
25	TA	53.0 ± 13.0 (44–80) [a,b]	19.0 ± 9.2 (8–40)	38.1 ± 0.6 (37.1–38.9)	94.8 ± 4.9 (85–100)	96.4 ± 15.0 (77–119)[a,b]
	TM	28.5 ± 10.3 (24–36)[a]	10.9 ± 4.1 (4–16)	38.8 ± 0.8 (37.5–39.9)	91.4 ± 9.2 (73–100)	112.9 ± 8.2 (102–123)[a]
	TMA*	34.0 ± 2.3 (32–36)[b]	13.5 ± 4.1 (10–18)	38.8 ± 0.6 (38.1–39.4)	87.8 ± 8.5 (81–100)	125.0 ± 9.0 (113–134)[b]
30	TA	53.0 ± 10.4 (44–72) [a,b]	20.3 ± 9.0 (12–40)[a]	38.0 ± 0.7 (36.8–38.8)	94.5 ± 3.6 (90–99)	95.9 ± 14.0 (81–124)[a,b]
	TM	31.3 ± 3.2 (28–36)[a]	11.1 ± 4.8 (4–18)[a]	38.6 ± 0.9 (37.3–39.9)	93.3 ± 5.6 (82–99)	114.0 ± 8.1 (103–123)[a]
	TMA*	35.0 ± 2.0 (32–36)[b]	13.0 ± 4.8 (8–18)	38.8 ± 0.6 (38.0–39.3)	87.5 ± 8.2 (80–99)	126.8 ± 11.0 (113–139)[b]
35	TA	55.5 ± 15.5 (44–88) [a,b]	18.8 ± 7.6 (12–36)	37.9 ± 0.7 (36.6–38.7)	96.3 ± 3.2 (90–99)[b]	97.6 ± 13.7 (82–123)[a,b]
	TM	32.0 ± 3.6 (28–36)[a]	11.4 ± 4.5 (4–16)	38.5 ± 0.9 (37.0–39.9)	94.0 ± 4.0 (89–99)	114.3 ± 7.9 (102–123)[a]
	TMA*	36.0 ± 0.0 (36–36) [b]	13.5 ± 6.4 (8–20)	38.6 ± 0.6 (37.9–39.1)	87.8 ± 9.3 (80–100)[b]	123.0 ± 8.5 (111–129)[b]

*Four animals required treatment with nasal oxygen after 15 minutes. Their data is not included after 15 minutes.
a: Significant difference between TA and TM, b: Significant difference between TA and TMA, c: Significant difference between TM and TMA ($\alpha \leq 0.05$).

Table II: Mean arterial blood variables ± SD (range) recorded during immobilisation of blesbok with TA (thiafentanil + azaperone), TM (thiafentanil + medetomidine) and TMA (thiafentanil + medetomidine + azaperone)

Time point (min)	Treatment	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	A-a gradient (mmHg)	Lactate (mmol/L)
10	TA	66.4 ± 5.8 (55.7–73.9)[b]	40.1 ± 2.7 (34.7–42.6)	29.9 ± 5.3 (22.6–39.1)[a,b]	0.91 ± 0.24 (0.54–1.36)
	TM	57.0 ± 7.5 (45.9–68.5)	38.2 ± 2.7 (34.0–41.4)[c]	41.2 ± 7.1 (29.8–51.7)[a]	0.65 ± 0.29 (0.15–1.13)
	TMA	41.2 ± 11.6 (27.4–64.5)[b]	43.1 ± 2.8 (38.1–46.4)[c]	53.0 ± 12.9 (30.1–73.2)[b]	0.93 ± 0.43 (.36–1.71)
15	TA	65.6 ± 7.2 (56.0–75.2)[a,b]	41.4 ± 2.4 (37.5–44.3)	29.4 ± 7.5 (18.6–38.5)[a,b]	0.82 ± 0.19 (0.54–1.18)
	TM	54.3 ± 6.5 (44.1–61.9)[a]	40.4 ± 4.1 (34.0–46.6)	41.7 ± 5.8 (34.5–52.7)[a]	0.68 ± 0.22 (0.42–1.03)
	TMA	38.9 ± 8.1 (27.7–55.4)[b]	44.9 ± 3.1 (39.5–48.9)	53.4 ± 10.4 (33.8–71.7)[b]	0.91 ± 0.40 (0.42–1.68)
25	TA	67.9 ± 7.3 (59.5–81.9)[a,b]	42.3 ± 4.2 (35.4–47.2)[b]	26.4 ± 6.4 (18.2–37.6)[a,b]	0.70 ± 0.13 (0.52–0.92)
	TM	55.1 ± 8.0 (41.9–68.4)[a,c]	43.0 ± 3.8 (38.3–47.6)[c]	38.3 ± 7.8 (25.2–52.2)[a]	0.68 ± 0.17 (0.46–0.92)
	TMA*	41.0 ± 5.5 (36.6–49.0)[b,c]	50.1 ± 1.6 (48.4–52.1)[b,c]	45.1 ± 5.0 (38.2–48.9)[b]	0.78 ± 0.25 (0.46–1.01)
35	TA	70.7 ± 6.0 (62.8–77.9)[a,b]	42.2 ± 3.5 (37.2–43.3)	23.8 ± 6.7 (14.7–33.8)[a,b]	0.66 ± 0.13 (0.48–0.88)
	TM	56.6 ± 6.6 (43.3–65.3)[a,c]	44.7 ± 7.8 (38.0–61.8)	35.3 ± 9.9 (16.4–48.6)[a]	0.69 ± 0.16 (0.52–0.93)
	TMA*	47.0 ± 3.9 (43.3–51.4)[b,c]	50.9 ± 0.6 (50.3–51.4)	38.6 ± 4.6 (32.9–42.2)[b]	0.72 ± 0.20 (0.52–0.97)

*Four animals required treatment with nasal oxygen after 15 minutes. Their data is not included after 15 minutes.
a: Significant difference between TA and TM, b: Significant difference between TA and TMA, c: Significant difference between TM and TMA ($\alpha \leq 0.05$).

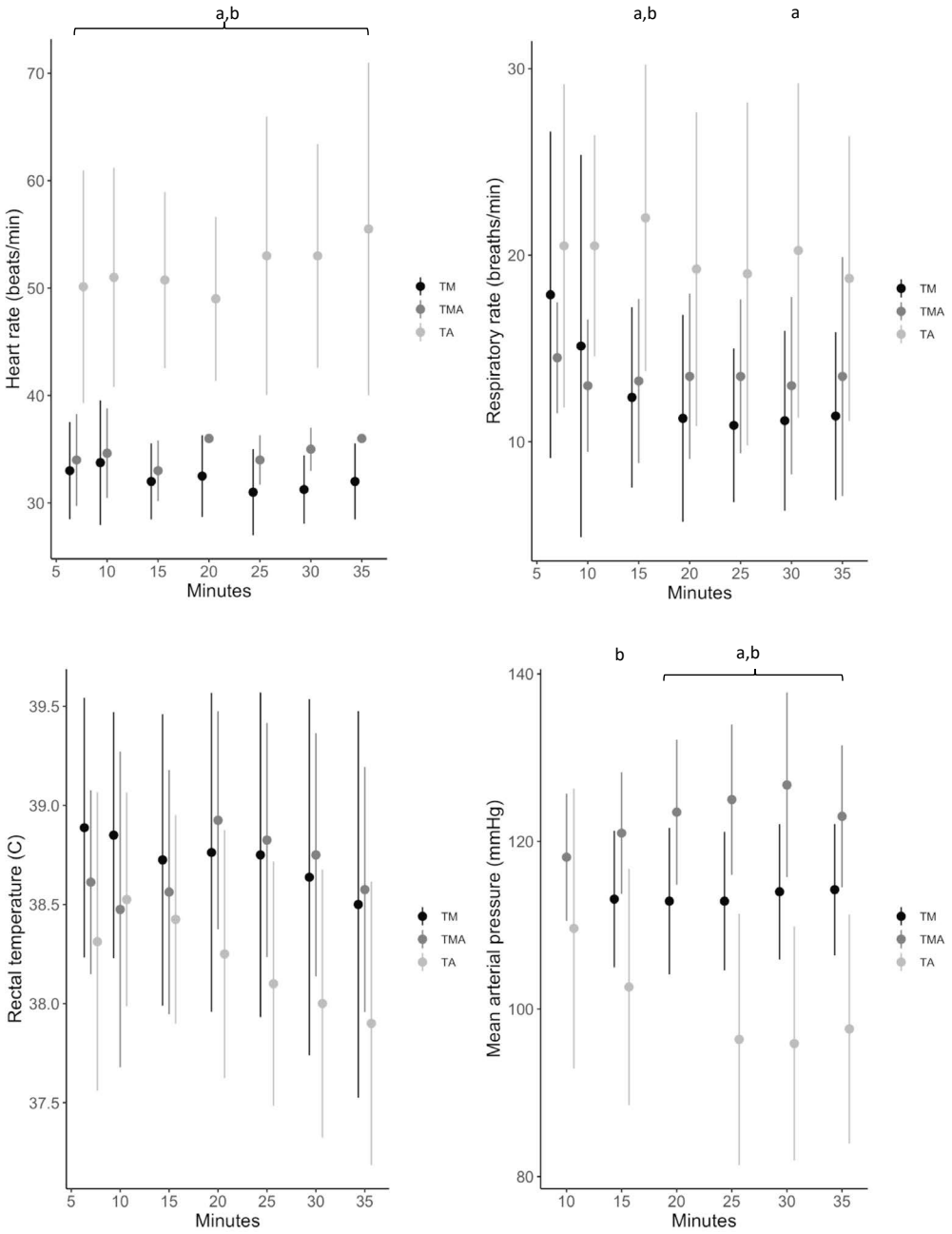

Figure 1: The mean and standard deviation of the heart rate, respiratory rate, rectal temperature, and mean arterial pressure of eight blesbok immobilised with three different drug combinations; TM = 0.5 mg thiafentanil + 1.5 mg medetomidine, TMA: 0.5 mg thiafentanil + 1.5 mg medetomidine + 10 mg azaperone, and TA: 2 mg thiafentanil + 10 mg azaperone. Four animals required treatment with intra-nasal oxygen after 15 minutes when immobilised with TMA. Their data is not included after 15 minutes. a: Significant difference between TA and TM, b: Significant difference between TA and TMA ($\alpha \leq 0.05$).

within 30 minutes when using TM despite good intramuscular dart placement, possibly because these two animals were more excited during the induction.

The TA combination resulted in the highest HR, RR, PaO_2, SpO_2, and lowest MAP and A-a gradients compared to both medetomidine combinations. The mean heart rate of conscious blesbok at rest was reported to range from 58–97 beats/minute while the mean respiration rate was reported to range from 13–17 breaths/min (du Plessis 2018). Medetomidine initially causes vasoconstriction and hypertension, followed by a slowing in heart rate caused by a baroreflex, and can cause a centrally mediated reduction in heart rate and ventilation (Posner 2018). Hypoxaemia is generally defined as a PaO_2 < 80 mmHg and an SpO_2 < 95%, and severe hypoxaemia is defined as a PaO_2 < 60 mmHg and an SpO_2 of less than 90% (Haskins 2015). Both the TMA and TM combinations therefore caused marked bradycardia and hypoxaemia, and four of the blesbok immobilised with TMA received intranasal oxygen which effectively corrected the hypoxaemia. Furthermore, there was a significant difference in PaO_2 between the TM and TMA combinations at some time points and it therefore appears that the hypoxaemia worsened with the addition of azaperone.

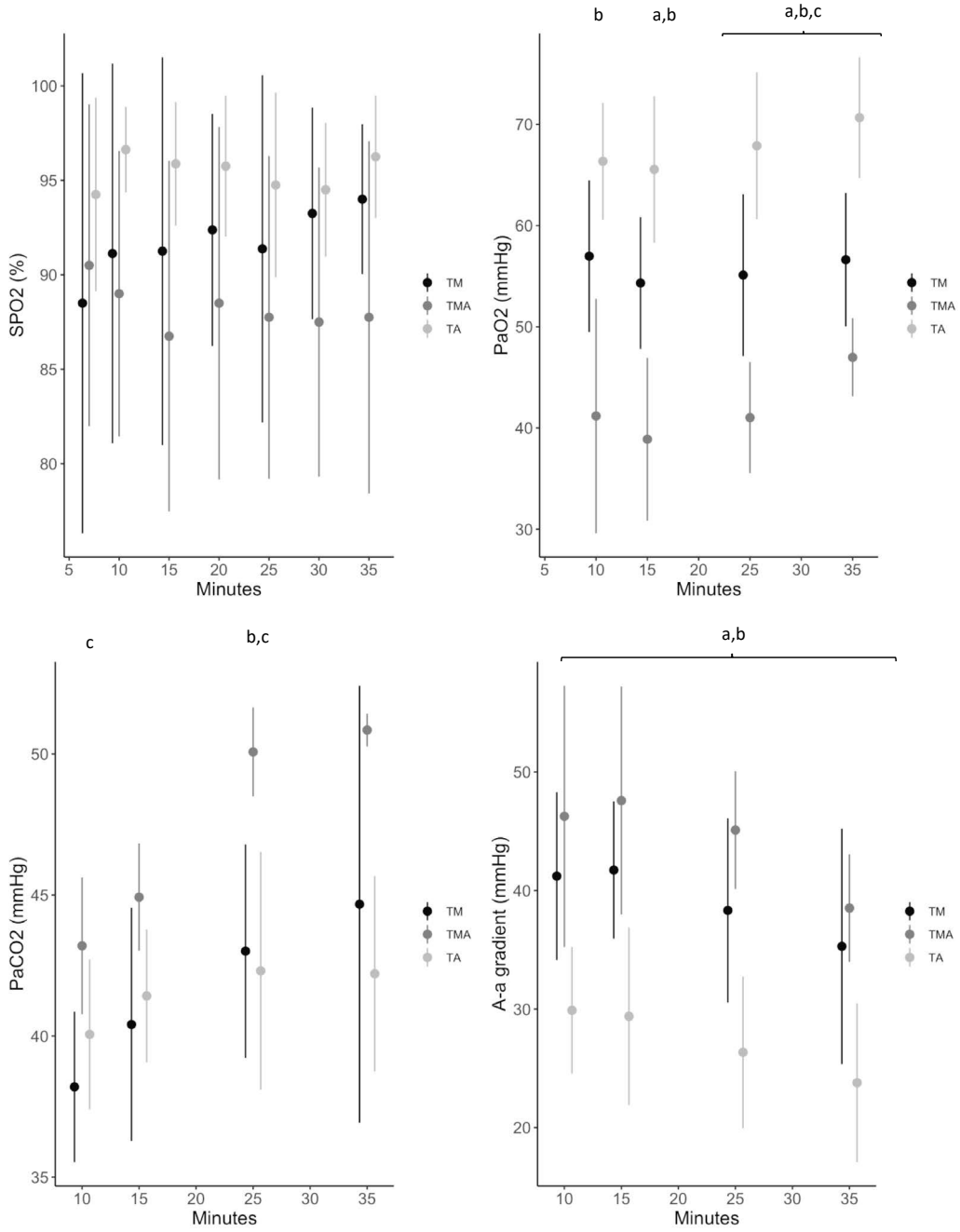

Figure 2: The mean and standard deviation of the sPO_2, PaO_2, $PaCO_2$, and A-a gradient of eight blesbok immobilised with three different drug combinations; TM = 0.5 mg thiafentanil + 1.5 mg medetomidine, TMA: 0.5 mg thiafentanil + 1.5 mg medetomidine + 10 mg azaperone. Four animals required treatment with intra-nasal oxygen after 15 minutes when immobilised with TMA. Their data is not included after 15 minutes. a: Significant difference between TA and TM, b: Significant difference between TA and TMA, c: Significant difference between TM and TMA ($\alpha \leq 0.05$).

Whether respiratory depression contributes to the hypoxaemia can be assessed by evaluating the $PaCO_2$. If ventilation is reduced, the ability to blow off excess CO_2 is impaired. The $PaCO_2$ in small ruminants is expected to be 40 mmHg +/- 7 mmHg (Ismail et al. 2010). The $PaCO_2$ values measured were highest in blesbok immobilised with TMA but were not severely elevated in any animal, indicating near adequate ventilation and riddance of CO_2 for all the combinations.

The cardiovascular effects of the $alpha_2$ adrenergic agonists may result in ventilation-perfusion mismatch in the lungs, which may reduce effective gas exchange (Read 2003; Kock & Burroughs 2021). The pulmonary effect of $alpha_2$ adrenergic agonists have not been evaluated in blesbok, but in sheep the $alpha_2$ adrenergic agonists cause congestion, and alveolar and interstitial lung oedema (Celly et al. 1997). In goats, potent opioids induced pulmonary hypertension, and the resulting pulmonary congestion and reduction in capillary blood transit time was reported to play a greater role in causing hypoxaemia than pure opioid-induced respiratory depression (Meyer et al. 2015; Pfitzer et al. 2020). The impaired gas exchange in the lungs induced mainly by the TM and TMA combinations, was reflected by the elevated A-a gradients. An elevated gradient indicates suboptimal alveolar-arteriolar oxygen transfer, which can be caused by pulmonary hypertension, congestion and alveolar-

interstitial oedema, or ventilation/perfusion mismatching (Haskins et al. 2015). A reference value for normal A-a gradients is not available for blesbok, but in small ruminants, a gradient of 20–25 mmHg is considered normal (Meyer et al. 2006). With the TA combination the mean A-a gradient ranged from 23.8–29.9, whereas with both the medetomidine combinations, the A-a gradients were elevated with means ranging from 35.3–41.7 mmHg for TM and 38.6–53.4 for TMA. We did not measure pulmonary vascular pressures and the exact cause of the elevated A-a gradient could therefore not be determined, but the deviation from normal indicated compromised gas exchange, especially when TM and TMA were used.

Several studies have measured physiological variables in blesbok immobilised with various drug combinations containing potent opioids, azaperone, and medetomidine. Pfitzer et al. (2021) immobilised blesbok with 0.09 mg/kg thiafentanil in the same bomas, and observed mean PaO_2 concentrations of 61–68 mmHg, which is higher than what we observed with the TM and TMA combinations where the thiafentanil dose was 0.01 mg/kg. Semjonov et al. (2018) evaluated the utility of butorphanol-azaperone-medetomidine (BAM) for immobilisation of blesbok, at the same location, and used a mean ± SD of 0.34 ± 0.08 mg/kg of butorphanol, 0.14 ± 0.03 mg/kg of azaperone, and 0.14 ± 0.03 mg/kg of medetomidine, and found a mean ± SD of PaO_2 of 72 ± 3 mmHg. In contrast, the dosage of medetomidine in our study was only 0.03 ± 0.003 mg/kg, but the addition of 0.01 ± 0.001 mg/kg thiafentanil and 0.18 ± 0.02 mg/kg azaperone significantly worsened the hypoxaemia compared to when immobilised with BAM. It is therefore possible that the severe hypoxaemia seen with TM and TMA is a result of a synergistic effect of the potent opioids, medetomidine and azaperone, and not a dose-dependent result of medetomidine, azaperone or thiafentanil alone.

The addition of azaperone shortened induction time compared to when using TM, and in contrast to TM, all blesbok became reliably immobilised with TMA with the first dart. In our study, the addition of azaperone to the TM combination appeared to intensify the hypoxaemia and possibly also lead to poorer ventilation as reflected in the lower PaO_2 concentration as well as higher $PaCO_2$ concentration at some time points. We did not observe lower blood pressures in blesbok immobilised with TMA in comparison to TM. The negative effect of azaperone on oxygenation is consistent with findings from a previous study that found that blesbok immobilised with etorphine-azaperone had lower PaO_2 and higher $PaCO_2$ values compared to when immobilised with etorphine alone (Gaudio et al. 2020). The respiratory depressive effect of azaperone should be kept in mind when using it in combination with potent opioids and $alpha_2$ adrenergic agonists.

Our data is limited by the small sample size. While all blesbok were healthy throughout the study, some observations may have been influenced more by individual characteristics of specific study animals. Four blesbok received intranasal oxygen after the 15 minute time point and their data from after the oxygen administration began was removed from the analysis. The oxygen administration was done for animal welfare reasons, but unfortunately also reduced our sample size further, making it impossible for us to assess whether the hypoxaemia would have worsened or corrected itself in these blesbok had we not administered oxygen. Further, treatment allocation was random; no attempt was made to distribute the different treatments evenly over the three immobilisation events. As a consequence, three animals were immobilised with TMA as their last treatment, and all three of these were provided oxygen due to low PaO_2 values. The remaining animal that received oxygen was immobilised with TMA as the second to last treatment. It is unknown whether there was some carry over effect from the repeated immobilisations, however, none of the animals immobilised with TM as the last treatment reached our threshold for oxygen administration. Additional studies comparing the physiological effects of the low-dose opioid combinations conducted in field settings, with larger sample sizes, may further elucidate other benefits and disadvantages of these drug combinations.

Conclusions

Blesbok immobilised with TA had higher heart rates, respiratory rates, PaO_2 and SpO_2 and lower MAP and $PaCO_2$ than blesbok immobilised with TM or TMA, who became bradycardic, and severely hypoxaemic. The addition of azaperone to the low-dose thiafentanil + medetomidine combination may have worsened the hypoxaemia, however the sample size is small. These results indicate that oxygen administration is highly recommended when using the low-dose thiafentanil + high-dose medetomidine combinations in blesbok.

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Competing interests

JP Raath, one of the investigators, has a financial interest in Wildlife Pharmaceuticals Pty Ltd, the company which also supported this project. However, we do not believe that this would have inappropriately influenced the analysis or writing of this article.

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Ethical considerations

All procedures performed in this study followed international, national, and/or institutional guidelines for the care and use of animals. The study was approved by both Wildlife Pharmaceutical's Animals Ethics Committee (Approval number: WPAEC-2022-VATINOXAN-51-B) and the University of Pretoria's Animal Ethics Committee (Approval number: REC083-21).

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