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Pharmacokinetics of intravenous propofol in southern white rhinoceros (*Ceratotherium simum simum*) after intramuscular etorphine-butorphanol-medetomidine-azaperone

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OBJECTIVE
To determine the pharmacokinetics of a single bolus of intravenous (IV) propofol after intramuscular administration of etorphine, butorphanol, medetomidine, and azaperone in 5 southern white rhinoceros to facilitate reproductive evaluations. A specific consideration was whether propofol would facilitate timely orotracheal intubation.

ANIMALS
5 adult, female, zoo-maintained southern white rhinoceros.

PROCEDURES
Rhinoceros were administered etorphine (0.002 mg/kg), butorphanol (0.02 to 0.026 mg/kg), medetomidine (0.023 to 0.025 mg/kg), and azaperone (0.014 to 0.017 mg/kg) intramuscularly (IM) prior to an IV dose of propofol (0.5 mg/kg). Physiologic parameters (heart rate, blood pressure, respiratory rate, and capnography), timed parameters (eg, time to initial effects and intubation), and quality of induction and intubation were recorded following drug administration. Venous blood was collected for analysis of plasma propofol concentrations using liquid chromatography-tandem mass spectrometry at various time points after propofol administration.

RESULTS
All animals were approachable following IM drug administration, and orotracheal intubation was achieved at 9.8 ± 2.0 minutes (mean ±SD) following propofol administration. The mean clearance for propofol was 14.2 ± 7.7 ml/min/kg, the mean terminal half-life was 82.4 ± 74.4 minutes, and the maximum concentration occurred at 2.8 ± 2.9 minutes. Two of 5 rhinoceros experienced apnea after propofol administration. Initial hypertension, which improved without intervention, was observed.

CLINICAL RELEVANCE
This study provides pharmacokinetic data and insight into the effects of propofol in rhinoceros anesthetized using etorphine, butorphanol, medetomidine, and azaperone. While apnea was observed in 2 rhinoceros, propofol administration allowed for rapid control of the airway and facilitated oxygen administration and ventilatory support.

The ongoing conservation efforts for the northern white rhinoceros (*Ceratotherium simum cottoni*) involve anesthesia for procedures including ovum harvesting from the southern white rhinoceros (*Ceratotherium simum simum*).[1] There are many unique anatomic and physiologic characteristics of the rhinoceros that make anesthesia challenging. Physiological changes during recumbency due to their size and drug-induced respiratory depression from the use of potent opioids contribute to hypercapnia and hypoxemia.[2–4] Intubation and mechanical ventilation have been shown to improve Pa_{O_2}, Pa_{CO_2}, and pHa during anesthetic recumbency in rhinoceros.[5] However, orotracheal intubation can be difficult in white rhinoceros due to their heavy head, narrow space between their dental arcades, and the

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presence of a dorsal pharyngeal recess. Muscle rigidity, often associated with the use of potent opioids, further complicates intubation.[2,6] While hydraulic wedge jacks have been used to help open the jaws to permit manual orotracheal intubation,[5,7] there are challenges with using this equipment, and delaying intubation can cause a significant compromise in an animal that may also be suffering from drug-induced respiratory depression during recumbency.

Based on literature supporting the use of propofol (2,6-diisopropylphenol), an injectable anesthetic used to facilitate reduced jaw tone for intubation,[8] the authors have explored the use of propofol in rhinoceros for this purpose. Preliminary work demonstrated that 0.5 mg/kg of propofol delivered intravenously (IV) shortened the time to intubation after remote intramuscular (IM) opioid and tranquilizer delivery as it allowed for the efficient opening of the jaw using polypropylene straps and manual traction rather than a hydraulic jack. Propofol was selected for its beneficial clinical characteristics, including a rapid onset and fast metabolic clearance.[9] In equids, which are relatives to rhinoceros, undesirable effects (eg, paddling, excitation, etc) are reported following propofol administered alone or in combination with an α_2-agonist.[10–13] Propofol can also cause respiratory depression, which may further compromise the rhinoceros during recumbency.

Prior to making a broad recommendation supporting the use of propofol in this species, the authors evaluated the pharmacokinetics, selected physiological parameters, and qualitative observations including the ease of intubation of a single dose of propofol in southern white rhinoceros. The objective of this study included evaluating the pharmacokinetics of propofol in the southern white rhino after induction with etorphine, butorphanol, medetomidine, and azaperone and evaluating the ability of propofol to help facilitate quick and uncomplicated intubation.

Materials and Methods

Animals

Five clinically healthy female southern white rhinoceros undergoing reproductive procedures as part of the northern white rhinoceros initiative were included in this study. Rhinoceros ranged in age from 7.0 to 23.5 years of age (13.7 ± 6.2 [mean ± SD]) and weighed between 1,620 to 2,075 kg (1,865.2 ± 185.1 kg). The pelleted diet (Grain-Free Enhancer Supplement for Elephants and White Rhino; Mazuri Exotic Animal Nutrition) was removed 18 hours prior to the procedures. Hay and browse removal were more variable pending animal temperament and housing conditions (range of 2 to 48 hours). Water was removed 2 hours before anesthetic drug administration via IM dart. The Institutional Animal Care and Use Committee at San Diego Zoo Wildlife Alliance approved the study.

Anesthesia

Rhinoceros were darted in the neck using a 3.0-ml plastic dart (DanInject LLC) with a 60-mm needle (DanInject LLC) using a compressed air rifle (DanInject LLC) by the attending veterinarian **(Figure 1)**. Animals received a combination of etorphine HCl (10 mg/ml; ZooPharm LLC; 0.002 mg/kg), butorphanol tartrate (30 mg/ml; ZooPharm LLC; 0.020 to 0.026 mg/kg), medetomidine HCl (20 mg/ml; ZooPharm LLC; 0.023 to 0.025 mg/kg), and azaperone (50 mg/ml; ZooPharm LLC; 0.014 to 0.017 mg/kg).

Figure 1—Southern white rhinoceros with appropriate dart placement within the musculature of the the lateral cervical region (photo credit: Kathryn Perrin, San Diego Zoo Wildlife Alliance).

The time when the dart contacted the animal was recorded as time 0. Time to first effects was recorded (typically high stepping, ataxia, or change in head position). When safe to approach the rhinoceros, a blindfold and earplugs were placed. Time to initial contact was recorded. With animals in sternal recumbency, propofol (10 mg/ml; Abbott Laboratories; 0.5 mg/kg) was administered in an auricular vein using a 19-G butterfly catheter with 1 exception in an animal that had an 18 g, 1-inch IV catheter placed in the auricular vein. After propofol was administered, the animals were moved into lateral recumbency onto cushioned pads for the duration of the procedure **(Figure 2)**. The timing and dose of propofol were extrapolated from equid species, prior case reports,[5–7] and the experiences of the authors.

A second veterinarian, referred to as the anesthetist, was responsible for intubation and ventilation throughout the procedure. Laterally recumbent rhinoceros were intubated using a 0.312 cm X 2-m polyoxymethylene stylet guide and a 26- or 30-mm internal diameter, 100-cm-long cuffed orotracheal tube (Smith Medical). Intubation was performed manually once polypropylene straps (183 cm X 5 cm) were positioned around the upper and lower jaw to open the mouth and provide space for a clinician to palpate the glottis. The polyoxymethylene stylet was then passed into the trachea via manual palpation. The stylet was passed through the Murphy eye of the endotracheal tube, and the tube was advanced over the stylet into the trachea, sometimes facilitated by

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Figure 2—Anesthetized and intubated southern white rhinoceros positioned in left lateral recumbency on large padding with anesthetic monitoring equipment (photo credit: San Diego Zoo Wildlife Alliance).

slight rotation as the tube was in proximity to the glottis. The stylet was then removed from the trachea, and endotracheal tube placement into the trachea was confirmed with end-tidal CO_2 measurements. Jaw tone was assessed on a 1 to 5 scale by the anesthetist intubating the rhinoceros, with 1 indicating no jaw tone and 5 indicating that the jaw tone was too significant to intubate. This assessment was based on the ease of introducing the forearm into the oral cavity and space available for manipulation of the glottis. Time to placement of the endotracheal tube from darting and end of propofol administration was recorded. Mechanical ventilation was initiated immediately after intubation using a mega-vertebrate demand ventilator (In Case of Anesthesia Wildlife Veterinary Specialists). The ventilator was powered by a 125-ft^3 tank (H cylinder) containing compressed medical oxygen with a drive gas pressure of 70 PSI, which generates a fraction of inspired oxygen (F_{IO_2}) of approximately 40%.[5] Inspiratory pressure and respiratory rate ranged from 26 to 32 cm H_2O.

An auricular venous catheter (18 or 20 gauge, 1.88 inch) was placed, and patency was maintained by the administration of lactated Ringer solution at a slow drip. A secondary catheter was placed into the cephalic vein (10 gauge, 2.5 inch) for additional IV fluids administration (Hartmann solution or lactated Ringer solution, total administration ranged from 4.5 to 10.3 ml/kg). Blood was sampled prior to fluid administration from 1 of the catheters (auricular or cephalic) for a complete blood count and serum chemistry.

Cardiovascular and respiratory parameters

Heart rate and rhythm (via electrocardiogram), end-tidal carbon dioxide, oxygen saturation, and direct arterial blood pressure were continuously monitored during recumbency using a multiparameter monitor, and values were recorded at 5-minute intervals once monitoring equipment could be safely placed. Direct blood pressure was similarly monitored and recorded following the placement of a 20-gauge, 1-inch medial auricular arterial catheter.

Blood gas analysis

The arterial catheter was also used to obtain blood for pH and blood gas analysis using an iSTAT blood gas analyzer and CG4+ cartridges (Abbott). Values were not corrected for body temperature. Arterial blood samples were collected every 20 to 30 minutes during recumbency. Hypercapnia was considered Pa_{CO_2} > 60 mmHg, hypoxemia was considered Pa_{O_2} < 90 mmHg, and respiratory acidosis was considered pH < 7.25.

Anesthetic recovery

Naltrexone (50 mg/ml; ZooPharm LLC; 0.05 to 0.06 mg/kg or 30:1 of etorphine:naltrexone) and atipamezole HCl (25 mg/ml; ZooPharm LLC; 0.12 to 0.15 mg/kg or 5:1 of medetomidine:atipamezole) were administered IM using an 18-gauge, 3.5-inch spinal needle in the lateral cervical region in the same muscles that were utilized for initial induction dart. Extubation was facilitated by the attending veterinarian and occurred after 2 to 3 spontaneous breaths were observed. All 5 rhinoceros were observed to move into sternal recumbency and then to a standing position. Times from darting to the administration of antagonists and from antagonist administration to extubation, initial arousal, head control, and standing were recorded.

Additionally, the overall anesthetic event including quality of induction and muscle relaxation was subjectively rated as "excellent, good, fair, or poor," and the recovery was rated as "normal or abnormal" by the attending veterinarian.

Procedures

Reproductive procedures included a rectal clean out of feces, rectal ultrasonography, and transrectal ovum pick-up. Ovum pick-up was performed using a previously published technique with some minor modifications. Briefly, after transrectal ultrasound identified an ovary, a double-lumen aspiration needle was used to enter multiple follicles, which were then aspirated and flushed multiple times to collect oocytes.[14]

Propofol sample collection

Blood was collected at 1, 2, 5, 15, 30, 60, 90, and 120 minutes after propofol administration. Actual sampling times were recorded. A blood sample was also collected immediately before antagonist administration if the timing did not coincide with a predesignated sampling point. Two of the 5 rhinoceros in this study were trained for voluntary venipuncture via behavioral restraint; these animals were sampled 24 hours postprocedure. The authors were unable to collect blood samples on the other 3 rhinoceros at

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this time point. Blood was collected via direct venipuncture of the ear using a butterfly catheter with or without a vacutainer or from the auricular venous catheter **(Figure 3)**. If taken from the catheter, scavenging methods were used, which included clamping the inferior vena cava line to stop venous flow and removing 5 to 10 ml of blood before taking a sample. Samples used for pharmacokinetic analysis were obtained from a different vein than the one used to administer the propofol. Blood samples were placed in 4-mL lithium heparin glass tubes or MiniCollect lithium heparin tubes (Greiner Bio-One) and centrifuged. Nonmicrotainer tubes were spun on the Thermo Scientific Sorvall ST16 at 4,100 rpm for 10 minutes. The microtainer tubes were spun on the Eppendorf 5417C at 14,500 rpm for 10 minutes. The plasma was collected and stored at −62.2 °C until analysis.

Figure 3—Venipuncture of the auricular vein of the southern white rhinoceros (photo credit: San Diego Zoo Wildlife Alliance).

Propofol plasma concentration determination

Plasma calibrators were prepared by dilution of the propofol working standard solutions (Cerilliant) with drug-free rhinoceros plasma to concentrations from 5 to 20,000 ng/ml. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality control samples (rhinoceros plasma fortified with analyte at 3 concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

Prior to analysis, 100 µL of plasma was diluted with 100 µL of acetone containing 1 ng/µL of D17-propofol internal standard (Cerilliant) to precipitate proteins. An additional 100 µL of acetone was added to all samples and methanol was added to bring the final volume to 350 µL. The samples were vortexed for 1 minute to mix, refrigerated for 20 minutes, vortexed for an additional minute, and centrifuged (4,300 rpm/3,102 X *g*) for 10 minutes at 4 °C, and 200 µL of the organic layer was transferred to a tube for derivatization. To the 200-µL sample, 100 µL of dansyl chloride (Sigma Aldrich), at 5 mg/mL in acetone, and 20 µL of 0.1 M sodium hydroxide were added. The samples were mixed briefly and heated at 60 °C for 10 minutes. Samples were transferred to an autosampler vial with an insert, and 10 µL was injected into the liquid chromatography-tandem mass spectrometry (LC-MS/MS) system.

The concentration of propofol was measured in plasma by LC-MS/MS using positive heated electrospray ionization [HESI(+)]. Quantitative analysis of plasma was performed on a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific) having an 1100 series liquid chromatography system (Agilent Technologies). The spray voltage was 3,500 V, the vaporizer temperature was 250 °C, and the sheath and auxiliary gas were 45 and 30, respectively (arbitrary units). Product masses and collision energies of each analyte were optimized by infusing the standards into the TSQ Vantage. Chromatography employed an ACE 3 C18 10 cm X 2.1-mm column (Mac-Mod Analytical) and a linear gradient of acetonitrile (ACN) in water with a constant 0.2% formic acid at a flow rate of 0.35 ml/min. The initial ACN concentration was held at 10% for 0.5 minutes, ramped to 98% over 6 minutes, and held at 98% for 0.67 minutes, before reequilibrating for 3.5 minutes at initial conditions.

Detection and quantification were conducted using selective reaction monitoring (SRM) of initial precursor ion for propofol (mass to charge [*m/z*] ratio, 412.2) and the internal standard D17-propofol (*m/z*, 429.2). The response for the product ions for propofol (*m/z*, 171.1, 156.1, and 115.1) and the internal standard D17-propofol (*m/z*, 171.1, 156.2) were plotted, and peaks at the proper retention time integrated using Quanbrowser software (Thermo Scientific). Quanbrowser software was used to generate calibration curves and quantitate propofol in all samples by linear regression analysis. A weighting factor of 1/X was used for all calibration curves.

Pharmacokinetic analysis

Noncompartmental analysis and a commercially available computer software program (Phoenix Winnonlin v8.3) were used for the determination of pharmacokinetic parameters. Actual, as opposed to nominal, sampling times and concentrations at or above the limit of quantitation (LOQ) were used for pharmacokinetic modeling. The area under the plasma concentration curve (AUC), the slope of the terminal portion of the concentration-time curve (λ_z), and terminal half-life (half-life λ_z) were determined. Lambda z was used to calculate the terminal half-life using the equation $0.693/\lambda_z$. The AUC from time 0 to infinity ($AUC_{0\rightarrow\infty}$) was determined using the linear up-log down trapezoidal rule and by dividing the last plasma concentration by λ_z extrapolated to infinity. Systemic clearance (Cl) and the apparent volume of distribution at steady state (V_{ss}) were determined using the following formulas:

$$Cl = Dose/AUC_{0\rightarrow\infty}$$

$$V_{ss} = MRT_{inf} \text{ X } Cl$$

where MRT_{inf} is the mean residence time to infinity.

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Results

Anesthesia

In all the procedures, the animal was approached following the administration of the drugs delivered by remote injection. No supplemental drugs were needed before propofol administration. Four of 5 animals became sternally recumbent, and the fifth was safely approached while still standing. A blindfold and body straps were placed over the neck and dorsum to move the rhinoceros away from the corner of the enclosure and into lateral and then sternal recumbency prior to propofol administration. One of the sternally recumbent animals was also moved using straps before propofol administration due to positioning in a corner of the habitat with poor access to the oral cavity for intubation.

Jaw tone during intubation was rated as a 1/5 for 1, 2/5 for 2, and 3/5 for the remaining 2 rhinoceros. For 1 of the 3/5 ratings, the clinician noted that it was slightly difficult to get their arm in at the rostral tip of the oral cavity compared to the other intubations performed during this study but was still successful. For 1 of the 2/5 ratings, the clinician noted that there was a loss of the lower jaw tone even when the bottom strap accidentally came out of the oral cavity during intubation.

The authors aimed to deliver the propofol over 60 seconds, but due to intermittent ear motion administration, the time varied between 35 to 152 seconds (mean, 68 seconds). Drug supplementation was needed to facilitate the completion of the procedure in 2 rhinoceros. In 1, a combination of etorphine (0.0002 mg/kg) and medetomidine (0.0058 mg/kg) was given IM 51 minutes after the initial dart, followed by 2 doses of IV ketamine (both 0.08 mg/kg) at 52 minutes and 59 minutes after the initial dart. The other rhinoceros was administered IV ketamine (0.087 mg/kg) and medetomidine (0.003 mg/kg) at 71 minutes and 76 minutes postinitial dart, respectively.

Timed qualitative parameters are included **(Table 1)**. Induction was rated as good in 4 animals and excellent in 1. Muscle relaxation was rated as excellent in 1 animal, good in 3, and fair in 1. Overall recovery following reversal administration was rated as "normal" for all 5 procedures. Mild ataxia of the left hind limb was documented in 1 rhinoceros, which resolved within a few hours without intervention.

Cardiovascular and respiratory parameters

Cardiovascular parameters measured during anesthetic maintenance were consistent with the values reported in previous rhinoceros studies using similar induction protocols and are reported **(Table 2)**. Heart rate was stable between 35 to 43 beats per minute for all 5 rhinoceros throughout the procedures. The ventilation rate was 5 to 12 breaths per minute. All 5 rhinoceros were initially hypertensive. Hypertension was defined as systolic > 160 mmHg or mean > 124 mmHg based on studies on rhinoceros in both awake and anesthetized settings.[15,16] The highest recorded systolic arterial pressure was 300 mmHg, and the highest mean arterial pressure was 261 mmHg; a gradual decrease was seen throughout the procedure for all 5 rhinoceros without intervention into a normal range. Two of the 5 rhinoceros had brief periods of apnea following propofol administration. One was intubated during the period of apnea, and 1 regained spontaneous respiration before intubation was complete.

Blood gas analysis

pH and blood gas values are summarized **(Table 3)**. Two rhinoceros demonstrated mild to moderate hypercapnia ($Paco_2$ > 60 mmHg) at 2 or more time points during recumbency. Blood lactate decreased over time in all 5 animals; the highest recorded value was 3.84 mmol/L. At certain time points, only 3 to 4 rhinoceros had blood gases reported; this was either due to an accidental venous sample collection, which was excluded from the analysis, or an issue occurred with the blood gas being run at that time (ie, machine or cartridge error).

Complete blood count and chemistries were within normal reference ranges reported for this species other than mild to moderate hyperglycemia

Table 1—Time of anesthetic milestones following intramuscular administration of etorphine-medetomidine-butorphanol-azaperone and subsequent intravenous administration of propofol (0.5 mg/kg) in 5 healthy southern white rhinoceros females.

Parameters	Rhino 1	Rhino 2	Rhino 3	Rhino 4	Rhino 5	Mean ± SD
Time to first effects (min)	3	4	3	1	3	2.8 ± 1.1
Time to recumbency (min)	6	11	5	8	17	9.4 ± 4.8
Time propofol given (min)	10	11	7	10	17	11 ± 3.7
Duration of propofol administration (s)	57	53	152	45	35	68.4 ± 47.5
Time to intubation (from dart) (min)	18	19	16	22	29	20.8 ± 5.1
Time to intubation (from propofol) (min)	8	8	9	12	12	9.8 ± 2.0
Time to extubation (from administration of reversal drugs) (min)	3	1	2	0	0	1.2 ± 1.3
Time to initial response (from administration of reversal drugs) (min)	11	5	3	8	6	6.6 ± 3.0
Time to head control (from administration of reversal drugs) (min)	12	6	15	9	7	9.8 ± 3.7
Time to standing (from administration of reversal drugs) (min)	12	7	15	10	7	10.2 ± 3.4

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Table 2—Range and median of selected cardiorespiratory parameters following intramuscular administration of etorphine-medetomidine-butorphanol-azaperone and subsequent intravenous administration of propofol (0.5 mg/kg) in 5 healthy southern white rhinoceros females.

Time from dart (min)	HR (beats/min)	SAP (mm Hg)	DAP (mm Hg)	MAP (mm Hg)	Ventilated respiratory rate (breaths/min)	Spontaneous respiratory rate (breaths/min)
10 to < 20						
Range	N/A	N/A	N/A	N/A	N/A	2–4
Median	N/A	N/A	N/A	N/A	N/A	3
20 to < 30						
Range	35–37 [b]	N/A	N/A	N/A	3–6	2
Median	36	N/A	N/A	N/A	5	2
30 to < 40						
Range	36–48	300[d]	201[d]	235[d]	3–6	N/A
Median	41	N/A	N/A	N/A	5	N/A
40 to < 50						
Range	35–42	285–300[b]	198–218[b]	228–261[b]	4	N/A
Median	40	300	198	236	4	N/A
50 to < 60						
Range	36–49	217–300	152–217	178–250	3–7	N/A
Median	42	300	177	210	4	N/A
60 to < 70						
Range	36–42	161–296	145–210	154–243	3–12	N/A
Median	41	263	187	205	5	
70 to < 80						
Range	36–41	114–300[a]	94–210[a]	101–245[a]	3–8	N/A
Median	40	237.5	159.5	196	5	N/A
80 to < 90						
Range	36–42	119–282[a]	93–201[a]	102–236[a]	3–7	N/A
Median	40	244	174.5	199.5	5	N/A
90 to < 100						
Range	36–42[a]	129–272[a]	88–184[a]	101–250[a]	3–10	N/A
Median	38.5	220	150	177	6	N/A
100 to < 110						
Range	35–43	124–252	102–173	104–211	3–7	N/A
Median	39	165	150	159	5	N/A
110 to < 120						
Range	38–44[a]	96–269[b]	68–188[b]	77–222[b]	3–11[b]	1–3[c]
Median	41	120	106	113	3	2
120 to < 130						
Range	36–40[c]	126[d]	108[d]	116[d]	6–10[c]	1–9[b]
Median	38	N/A	N/A	N/A	8	6
130 to < 140						
Range	36–38[c]	122[d]	108[d]	116[d]	6[c]	10[d]
Median	37	N/A	N/A	N/A	6	N/A
140 to < 150						
Range	40[c]	115[d]	100[d]	107[d]	2–5[c]	N/A
Median	40	N/A	N/A	N/A	3.5	N/A
> 150						
Range	40[d]	N/A	N/A	N/A	N/A	4–5[c]
Median	N/A	N/A	N/A	N/A	N/A	4.5

Controlled respirations = the rate of manual ventilation (breaths/minute). DAP = Diastolic arterial pressure (mmHg). HR = Heart rate (beats/minute). N/A = Not available. MAP = Mean arterial pressure (mmHg). SAP = systolic arterial pressure (mmHg). Spontaneous RR = Rhinoceros' spontaneous respiratory rate (breaths/minute).

[a–d]Superscript next to the range for each parameter indicates the total number of rhinoceros included in the data set (a = 4; b = 3; c = 2; and d = 1). If no superscript is present, all 5 rhinoceros included.

Reported blood pressures were all recorded from invasive blood pressure monitoring.

(176 to 250 mg/dL; mean, 215.4 ± 29.5 mg/dL) noted in all 5 animals.

Assay performance

The response was linear and gave correlation coefficients of 0.99 or better. The precision and accuracy of the assay were determined by assaying quality control samples in replicates (n = 6) for propofol. Accuracy was reported as percent nominal concentration and precision as percent relative standard deviation. For propofol, accuracy was 99% for 7.5 ng/mL, 108% for 250 ng/mL, and 110% for 5,000 ng/mL. Precision was 12% for 7.5 ng/mL, 7% for 250 ng/mL, and 5% for 5,000 ng/mL. The method was optimized to provide a limit of quantitation of 5 ng/mL and a limit of detection of approximately 2.5 ng/mL.

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Table 3—Mean ± SD of pH, lactate, and arterial blood gas values at various time points following intramuscular administration of etorphine-medetomidine-butorphanol-azaperone and subsequent intravenous administration of propofol (0.5 mg/kg) in 5 healthy southern white rhinoceros females (n = 4 for each time point except where indicated).

Time from induction (min)	pHa	$Paco_2$	Pao_2	Lactate
15–25 min				
Range	7.32–7.35	47.8–54.1	57–187	3.26–3.84
Mean ± SD	7.33 ± 0.01	50.47 ± 3.26	107.33 ± 69.79	3.56 ± 0.29
26–40 min				
Range	7.35–7.44	42.3–60.2	112–170	1.52–2.79
Mean ± SD	7.43 ± 0.08	45.03 ± 12.15	148.5 ± 27.36	1.89 ± 0.77
41–60 min				
Range	7.33–7.43	48.7–54.6	92–163	1.61–2.4
Mean ± SD	7.38 ± 0.04	50.8 ± 2.69	129 ± 31.36	2.19 ± 0.39
61–80 min				
Range	7.36–7.47	38.1–59	84–205	1.38–2.3
Mean ± SD	7.39 ± 0.05	50.08 ± 8.77	139.75 ± 50.13	1.95 ± 0.4
81–101 min				
Range	7.26–7.47	42.9–78.9	101–172	1.52–2.12
Mean ± SD	7.37 ± 0.08	55.78 ± 15.87	137.5 ± 29.01	1.85 ± 0.25
102–125 min (n = 3)				
Range	7.26–7.39	50.6–71.9	81–177	0.96–1.7
Mean ± SD	7.32 ± 0.07	61.9 ± 10.71	128.67 ± 48	1.36 ± 0.37

Pharmacokinetic results

Actual blood collection times were slightly varied based on the accessibility of the vein during the procedure and total length of the procedure. Samples for 1 and 2 minutes were lost for 1 rhinoceros and for 90 and 120 minutes for another rhinoceros. The mean plasma propofol concentrations and individual plasma concentrations for each individual rhinoceros are shown **(Figure 4)**. In 4 of the 5 animals, the initial sample had the highest concentration of propofol (1.5 minutes, 7.67 minutes, 0.92 minutes, and 0.88 minutes). In the other rhinoceros, the peak plasma concentration was noted at 3.13 minutes (the second or intended 2-minute time sample) from the administration of propofol. Of the 2 animals sampled at 1,440 minutes postpropofol administration,

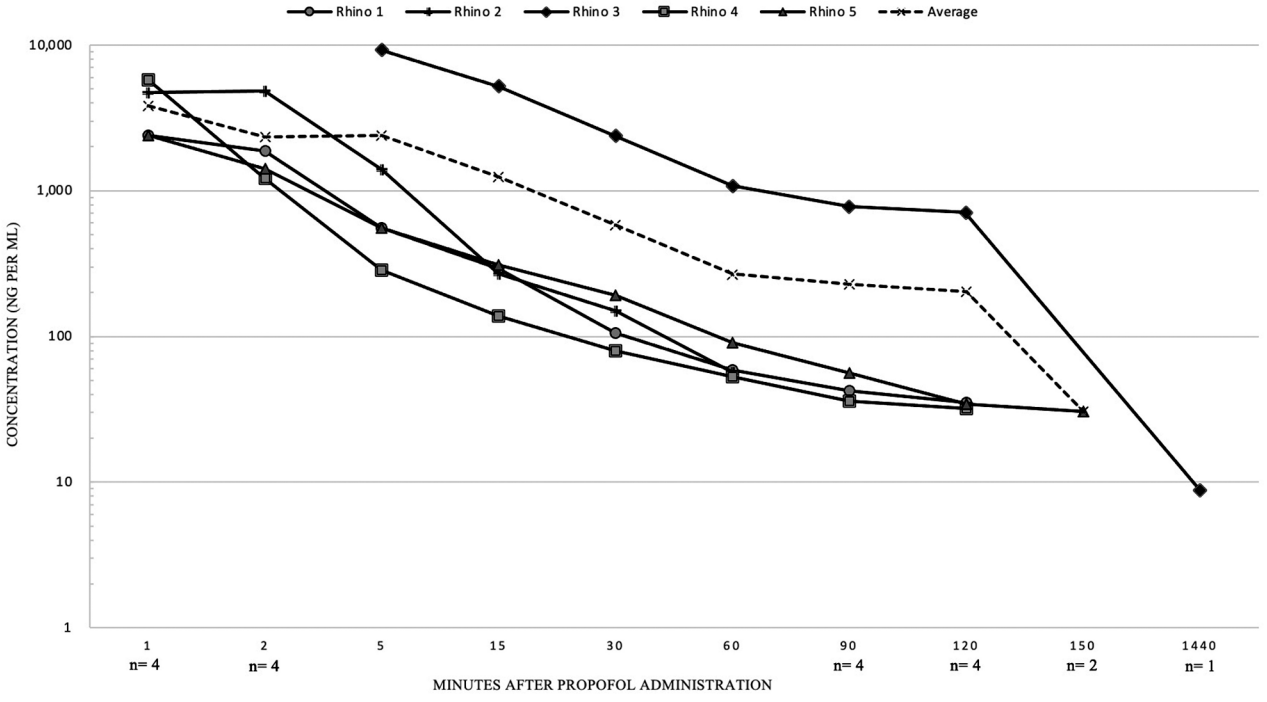

Figure 4—Average and individual plasma concentrations (ng/ml) of propofol over time for 5 southern white rhinoceros following intravenous propofol (0.5 mg/kg, IV) administered to 5 healthy female southern white rhinoceros after receiving intramuscular etorphine-medetomidine-butorphanol-azaperone; n = 5 for number of samples at the given time, unless indicated.

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1 animal had levels below the detectable range, and 1 animal had a concentration of 8.75 ng/ml.

Pharmacokinetic parameters (mean ± SD) are reported **(Table 4)**. Propofol concentrations declined rapidly in 4 of the 5 rhinoceros, with half-lives ranging from 20 to 80 minutes, and 1 outlier with a longer half-life of 210 minutes. This animal also had the highest peak concentration of propofol, the lowest clearance, and the longest time for propofol administration (152 seconds).

Table 4—Mean ± SD pharmacokinetic parameters following intravenous propofol (0.5 mg/kg, IV) administered to 5 healthy female southern white rhinoceros after receiving intramuscular etorphine-medetomidine-butorphanol-azaperone.

Parameters	Mean ± SD
AUC_{extrap} (%)	7.50 ± 5.51
AUC_0-∞ (min·ng/mL)	124,499.9 ± 213,170.8
Terminal half-life (HL λ_z) (min)	82.4 ± 74.4
λ_z (1/min)	0.015 ± 0.012
C_0 (ng/mL)	8,645.9 ± 6,440.6
Cl (mL/min/kg)	14.2 ± 7.7
V_{ss} (mL/kg)	535.5 ± 357.7

All values were generated using noncompartmental analysis.

λ_z= Terminal slope. AUC_{extrap} = Percentage of area under the concentration versus time curve extrapolated. AUC_0-∞ = Area under the plasma concentration curve from time 0 to infinity. C_0 = Concentration at time 0. Cl = Clearance. HL λ_z = Terminal half-life. V_{ss} = Steady-state volume of distribution.

Discussion

This study describes the pharmacokinetics of a single IV propofol bolus following IM administration of a combination of etorphine, butorphanol, medetomidine, and azaperone in southern white rhinoceros.

The use of a potent opioid (etorphine), opioid agonist-antagonist (butorphanol), α_2-agonist (medetomidine), and a neuroleptic agent (azaperone) in combination is a well-described method of inducing recumbency in southern white rhinoceros.[4,5] However, in author and additional veterinarian experiences, additional supplementation may be necessary to enhance anesthetic depth and facilitate timely intubation. In this study, propofol was administered to all 5 rhinoceros to facilitate movement from sternal to lateral recumbency and to provide adequate muscle relaxation to allow for intubation without the use of a hydraulic jack. The manual method of opening the jaws requires less technical knowledge and minimizes potential injuries to the oral cavity of the rhinoceros compared to hydraulic jack-assisted intubation. The impact of propofol administration on breathing was manageable in the 2 rhinoceros that became apneic after propofol administration. One regained spontaneous respiration before intubation, and the other was intubated and ventilated without complications.

The hyperglycemia observed during this study is consistent with the use of α_2-adrenergic agonists; however, the authors cannot rule out stress from the movement before the procedure or from darting as a contributing factor.[17] The only significant physiological abnormality was the hypertension observed in all animals in this study. This is consistent with previous reports[16,18]in horses and rhinoceros receiving etorphine. Hypertension decreased throughout the procedure into normal range without intervention and no overt adverse effects were noted. There was increased $Paco_2$ at various time points for 4 of the 5 rhinoceros. The results were compared to values from both anesthetized and awake captive rhinoceros.[5,15] In this study, likely due to intermittent mechanical ventilation, hypercapnia ($Paco_2$ > 60 mmHg) and hypoventilation were less common and less severe compared to previous studies, where these were consistent findings.[4] The lowest mean pH for any time point in this study was 7.32, with the highest mean $Paco_2$ of 61.9 mm Hg. Despite ventilation, 1 animal was consistently hypercapnic toward the end of the procedure. While the reasons are not known, it is possible that as adjunctive drug effects were wearing off, she had increased chest wall rigidity. She was also noted to be spontaneously breathing intermittently, which may also have interfered with her ability to ventilate effectively. As animals were allowed access to hay for a period of time prior to anesthesia, distention of the gastrointestinal tract may also have contributed. If normalization of ventilation is critical under these circumstances, the use of supplemental drugs might be indicated.

Several propofol pharmacokinetic studies have been performed in horses, which are an anatomically and physiologically similar domestic species to the rhinoceros. When compared to a study in horses evaluating a single bolus of propofol at 2 mg/kg, the half-life was shorter in horses (44.8 ± 21.3 minutes) and the clearance was faster (45.8 ± 6.5 ml/min/kg) than the present study, with a half-life and clearance of 82.4 ± 74.4 minutes and 14.2 ± 7.7 ml/min/kg, respectively.[19] In another study[20] in ponies, a 0.5 mg/kg bolus of propofol was given IV, followed by a 60-minute infusion of propofol and ketamine. The half-life was shorter (69 ± 8.0 minutes), and the clearance was faster (33.1 ± 4.5 ml/min/kg) than in the rhinoceros in the present study.[20] The clearance in the present rhinoceros study is also slower than values reported for dogs, sheep, and goats again highlighting species differences.[21–23] Other potential reasons for differences between the current and prior propofol studies in other species may be related to dosage and differences in concurrent medications. It is noteworthy that studies[24] have shown significant variation in pharmacokinetics between the horse and rhinoceros with the rhinoceros exhibiting slower clearance of carprofen and enrofloxacin. The reasons are not known, but a difference in the CYP450 enzyme genome, an important enzyme for propofol metabolism, may explain the differences in the metabolism of drugs.[25,26]

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While the logistics and risks of anesthetizing mega-vertebrates result in inherent limitations, this study was unique in that authors were able to perform the same procedure on 5 rhinoceros in a controlled setting with the same initial drug protocol, reducing aspects of variability between animals. Due to logistical considerations, a blood sample prior to drug administration could not be obtained. However, the most recent anesthesia for all rhinoceros in the study was 2 years prior, so it is unlikely there were circulating concentrations of propofol before sampling for this study. While attempts were made to administer propofol consistently, the rate of propofol administration varied based on challenges with rhinoceros positioning, access to the auricular vein, and intermittent ear movement. This led to 1 animal having propofol administered over 152 seconds, compared to the other 4 rhinoceros receiving propofol 0.5 mg/kg over 35 to 57 seconds. Interestingly, the rhinoceros that received propofol over the longer injection time had the highest concentration of propofol in the study, as well as the slowest clearance and longest half-life.

This study describes the drug disposition following a single IV dose of 0.5 mg/kg propofol after IM etorphine, butorphanol, medetomidine, and azaperone in southern white rhinoceros. Intubation was facilitated by the administration of propofol, which allows for earlier management of ventilation and oxygen supplementation. Given that apnea is possible following propofol administration, its use at this dose should be limited to circumstances where respiratory supportive measures are available.

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