

A study of the pharmacokinetics and thromboxane inhibitory activity of a single intramuscular dose of carprofen as a means to establish its potential use as an analgesic drug in white rhinoceros

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Funding information

Zoetis; South African Veterinary Association (SAVA); Bayer Animal Health; Deutscher Akademischer Austauschdienst; University of Pretoria

The alleviation of pain and prevention of suffering are key aspects of animal welfare. Unfortunately, analgesic drugs are not available for all species. White rhinoceros (*Ceratotherium simum*), representing one of such species, which survive poaching attempts inflicted with severe facial injuries and gunshot wounds, nonetheless require analgesic support. To improve treatment conditions, this study explored the use of carprofen for the treatment of pain and inflammation in white rhinoceros. The pharmacokinetics of 1 mg/kg intramuscular carprofen was evaluated in six healthy white rhinoceros. The half-life of λ_z and mean residence time was 105.71 ± 15.67 and 155.01 ± 22.46 hr, respectively. The area under the curve and the maximum carprofen concentration were $904.61 \pm 110.78 \mu\text{g ml}^{-1} \text{hr}^{-1}$ and $5.77 \pm 0.63 \mu\text{g/ml}$, respectively. Plasma TXB_2 inhibition demonstrated anti-inflammatory properties and indicated that carprofen may be effective for a minimum of 48 hr in most animals. With its long half-life further indicating that a single dose could be effective for several days, we suggest that carprofen may be a useful drug for the treatment of white rhinoceros.

1 | INTRODUCTION

Pain has been described as “a more terrible lord of mankind than even death itself” by Dr. Albert Schweitzer. However, despite the recent recognition of pain in animal species and the now widely accepted importance of pain management in veterinary medicine, not all veterinary patients are easily treatable. The southern white rhinoceros (*Ceratotherium simum simum*), one of such species, has only recently come to the forefront as a species requiring appropriate analgesic treatment, all as a result of poaching and the illegal acquisition of rhino horn (Emslie et al., 2016). In most cases, this results in the brutal killing of the animals (Emslie et al., 2016), however, some individuals miraculously survive the attack and are found alive often suffering from tremendous facial injuries, bone

loss and severe haemorrhaging. Roughly, 200 animals per year are in need of veterinary assistance due to poaching and poaching-related injuries (personal communication, Dr. Johan Marais, Saving the Survivors). Besides the facial wounds, injuries encountered in those animals include gunshot wounds and wounds caused by snares (Mulero-Pazmany, Stolper, van Essen, Negro, & Sassen, 2014; Streicher, Newcomer, McCarty, & Brook, 2010). To alleviate pain and reduce the suffering of those individuals, appropriate analgesic treatment is needed. Amongst the different pain relieving drugs in animal species, NSAIDs are one of the most widely used for perioperative analgesia in horses, cats and dogs, as well as for chronic pain relief in osteoarthritis in dogs (Lees, Aliabadi, & Landoni, 2002; Lipscomb, AliAbadi, Lees, Pead, & Muir, 2002; Schatzmann, Gugelmann, Voncranach, Ludwig, & Rehm, 1990). NSAIDs are also

readily applied in cattle for the reduction of acute inflammatory reaction in respiratory diseases (Balmer, Williams, & Selman, 1997) and further for the treatment of peracute and acute bovine mastitis (Papich, 2010).

For this study, carprofen (± 6 -chloro- α -methyl-carbazole-2-acetic acid), a propionic acid derivative, was chosen for further evaluation as a potentially effective and appropriate NSAID for the use in white rhinoceros. The choice was influenced by wild-life veterinarians who reported obtaining satisfactory analgesia for 2–3 days following the administration of carprofen to rhino suffering from lameness (personal communication, Dr. J. Marais, Saving the Survivors). Furthermore, carprofen is defined by its low incidence of side effects and high therapeutic index in most veterinary species, with the possible exception of the vulture (Fourie, Cromarty, Duncan, Wolter, & Naidoo, 2015). The wide safety margin seen in most species may be explained by the relatively weak to moderate inhibition of the COX-1 and COX-2 isoforms observed in species like the horse (Lees et al., 2002), calf (Delatour, Foot, Foster, Baggot, & Lees, 1996), cat (Taylor et al., 1996) and dog (McKellar et al., 1990) given at clinically recommended drug doses. Furthermore, carprofen exhibits excellent analgesic properties for soft tissue injuries, perioperative pain management as well as after orthopaedic procedures (Grisneaux, Pibarot, Dupuis, & Blais, 1999; Mathews, 2002) and can be administered intravenously (i.v.) intramuscularly (i.m.) as well as subcutaneously (s.c.) and per os (p.o.; McKellar, Bogan, von Fellenberg, Ludwig, & Cawley, 1991; McKellar, Delatour, & Lees, 1994; Taylor et al., 1996). Carprofen is characterized by a low volume of distribution in steady-state of 0.093 L/kg in sheep (Welsh, Baxter, & Nolan, 1992), 0.14 L/kg in the dog (McKellar et al., 1990) and 0.091 L/kg in the cow (Lohuis et al., 1991). Furthermore, it is known for its long half-life of elimination in the horse (21.9 hr; McKellar et al., 1991), the cow (30.7 hr; Lohuis et al., 1991) and sheep (26.1 hr; Welsh et al., 1992). The clearance ranges between 0.75 ml kg⁻¹ hr⁻¹ in horses (McKellar et al., 1991), 2.4 ml kg⁻¹ hr⁻¹ in cattle and 2.5 ml kg⁻¹ hr⁻¹ in sheep (Welsh et al., 1992). When looking at the different enantiomers of the chiral drug carprofen as opposed to the total carprofen concentration, the R(-) carprofen plasma concentrations exceed those of the S(+) carprofen. Furthermore, in species such as the horse, the mean residence time and elimination half-life have been found to be three times longer for R(-) carprofen than for S(+) carprofen when administered at 0.7 mg/kg, while the clearance of the S(+) carprofen was 5.7 times more rapid (Lees et al., 2002). The enantiomer concentrations were found to increase in direct proportion to the administered dose. Even though quantitatively the R(-) enantiomer is predominant, the effect of racemic carprofen can be almost completely ascribed to the S(+) enantiomer (Lees, Giraudel, Landoni, & Toutain, 2004), which is characterized by a significantly higher potency.

Due to the complexity of working with wild, endangered animals, we evaluate carprofen as it would be administered in clinical practice, which is in conjunction with an antimicrobial, while the animals are immobilized.

2 | MATERIALS AND METHODS

2.1 | Animals

The study proposal was reviewed and approved by the Animal Ethics Committee of the University of Pretoria (Approval number: V074-15). Six white rhinoceros, one female and five males from “The Rhino Orphanage” in South Africa, were included in the study. The animals were between 15 and 30 months old, weighing in average 670 (538–902) kg. The detailed characteristics of the rhinoceros are summarized in Table 1. Details on the living conditions, daily feeds and the habituation of the animals to the touching of their ears are presented in a previous study on the pharmacokinetics of the concurrently administered enrofloxacin.

2.2 | Experimental design and sampling procedure

Six white rhinoceros received a single i.m. injection of racemic carprofen (± 6 -chloro- α -methyl-carbazole-2-acetic acid) at 1 mg/kg (Rimadyl Injectable Solution, 50 mg/kg, Zoetis) into the gluteal muscle. No more than 18 ml of carprofen was injected at one injection site. Carprofen was administered in conjunction with a single dose of i.v. enrofloxacin (12.5 mg/kg, Baytril, Injectable, Bayer Animal Health, 100 mg/ml), followed by a subsequent dose of oral enrofloxacin (12.5 mg/kg, Baytril, Bayer Animal Health, 10% oral solution) after 10.45 \pm 0.76 hr (enrofloxacin results not included in this publication). Enrofloxacin was evaluated in combination with carprofen as many wounds require concurrently antimicrobial therapy. The study of enrofloxacin at the same time thus simulated not only expected clinical use but also allowed for the maximum use of the study animals, which are not easy to source and work with.

The carprofen and thromboxane B₂ (TXB₂) plasma concentrations were evaluated over a period of roughly 72 hr from a maximum of 11 blood samples per animal. The blood samples were taken prior to the drug administration and in average after 4.68, 18.45, 31.1, 45.77 min and 2.11, 6.17, 11.87, 22.8, 47.65, 73.07 hr postcarprofen injection. A total of 18 ml of blood was collected in lithium heparin tubes and partly used for the analysis of the carprofen plasma concentration. Additional 2–4 ml of blood was collected in indomethacin spiked serum tubes for the measurement of plasma thromboxane B₂ (TXB₂) activity. For the indomethacin treatment of the tubes, 0.05 ml of a 0.04 M indomethacin solution and 0.95 ml of the EDTA

TABLE 1 Characteristics of the six white rhinoceros used in the study listed according to their age

Rhino	Age (months)	Sex	Weight (kg)
Rhino I	15	Female	556
Rhino II	15	Male	538
Rhino IV	19	Male	551
Rhino III	20	Male	573
Rhino V	30	Male	900
Rhino VI	30	Male	902

buffer (pH of 7.4) were mixed for every 10 ml of blood. Syringes and vacutainer tubes were coated according to their appropriate volume. For the EDTA buffer, 2 g disodium EDTA (purity >99%, Sigma Aldrich) and 0.8 g sodium chloride (purity >99%, Merck) were mixed with distilled water to a total volume of 100 ml and adjusted with NaOH (purity 98%, UnivAR) to the pH of 7.4. The 0.04 M indomethacin solution consisted of 50 mg of indomethacin (purity ≥99% [TLC], Sigma Aldrich) dissolved in 3.5 ml absolute ethanol (purity 99.9%, ILLOVO).

2.2.1 | Immobilization of the rhinoceros

The placement of the auricular catheter, the administration of the drugs and the first five blood samples were not possible without chemical immobilization of the animals. Thus, the six rhinoceros were sedated using a mixture of etorphine (M99, 9.8 mg/ml, Novartis) and thiafentanil (Thianil, 10 mg/ml, Wildlife Pharmaceuticals RSA). All rhinos also received 50 mg of zuclopenthixol acetate (Clopixol-Acuphase, 50 mg/ml) within 2.5 hr of the initial immobilization. Diprenorphine (M5050, 12 mg/ml, Novartis) was given at a dose of 0.2 or 0.5 mg during the immobilization to improve the breathing and at a higher dose of 5 mg per animal at the end of the immobilization as a partial reversal, if necessary. Additionally, medetomidine (20 mg/ml, Kyron RSA) and ketamine (50 mg/ml, Kyron RSA) were used to improve anaesthetic depth when needed. The details of the sedation of each individual are summarized in Table S1. The blood samples scheduled at 24, 48 and 72 hr were collected under a low-dose butorphanol (Dolorex, MSD animal health, 10 mg/ml) based standing sedation directly from the cephalic vein. The details regarding the butorphanol based low-dose sedation are summarized in Table S2.

2.3 | Analytical techniques

2.3.1 | Processing of the blood samples

All samples were placed on ice immediately after blood collection and centrifuged within 4 hr at 503 g for 15 min. Plasma samples for pharmacokinetic evaluation were frozen at -20°C for a maximum of 8 days prior to storage in a -80°C freezer. The blood collected in indomethacin spiked serum tubes for the pharmacodynamic evaluation of carprofen was flash-frozen in liquid nitrogen and transferred to the -80°C freezer after a maximum of 8 days. Analysis of the Carprofen Plasma Concentrations via Tandem Mass Spectrometry–Liquid Chromatography.

The samples for the pharmacokinetic analysis of carprofen were shipped to Germany (dry ice, World Courier) for analysis by Bayer Animal Health (CITES export permit number: 152722) and analysed by high-performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS).

The plasma samples were mixed with the internal standard carprofen- d_3 and deproteinized. Therefore, 100 μl of plasma was added to 900 μl of a mixture containing 100 ml of 0.40 g ammonium acetate in 1L water, 1 ml formic acid and 600 ml acetonitrile. The

samples were placed on a vibratory mixer for 30 s prior to centrifugation. An aliquot of 5 μl was then injected into the HPLC system. The chromatographic separation was performed with a ZORBAX SB Phenyl column, 2.1 \times 50 mm, 1.8 μm (Agilent Technologies, Waldbronn, Germany) using the mobile phase A (water, 10 mM ammonium formate and 0.12 ml/L formic acid) and the mobile phase B (methanol, 10 mM ammonium formate, and 0.12 ml/L formic acid). Gradient elution with a flow rate of 0.5 ml/min started at 10% B for 0.5 min, eluent strength was then increased and, after 1.5 min, reached 95% B for 1 min. Thereafter, the eluent strength decreased to 10% B after 3 min and was equilibrated for 1 min. The quantitative determination was performed by HPLC with detection by tandem mass spectrometry using an API 5500 mass spectrometer (Sciex, Darmstadt, Germany). The retention time for carprofen was 1.7 min. Carprofen was detected in the positive ionization mode using the transition from its precursor ion at m/z 274 to its product ion at m/z 228 or m/z 193. The internal standard carprofen- d_3 was detected in the positive ionization mode using the transition from its precursor ion at m/z 277 to its product ion at m/z 231. The limit of quantification was 0.01 mg/L. As the amount of available rhino control plasma was limited, the method validation was performed using four validation levels (0.01/0.1/1/10 $\mu\text{g/ml}$) with three replicates each. The mean recovery rates for the individual fortification levels were between 81% and 97%. The mean overall was 93%, representing an accuracy of -7% with a precision (coefficient of variation) of 10%. The method is a variation of our routine plasma method and shows a high specificity as a result of the selective determination by tandem mass spectrometry. Matrix effects are compensated by the use of an internal standard. The limit of detection was set to the lowest standard concentration used for the measurement, which corresponds to 0.005 $\mu\text{g/ml}$.

2.3.2 | Thromboxane B2 analysis

The inhibition of TXB_2 , the stable metabolite of thromboxane A_2 (TXA_2), was used as an indicator for the extent and the time-dependent course/duration of COX-1 inhibition (Lees et al., 2002). The “Thromboxane B2 Express EIA Kit - Monoclonal” (Cayman Chemical) was used to measure the TXB_2 concentrations in the plasma. Plasma samples were purified prior to TXB_2 analysis via ELISA, and all procedures were carried out according to the instructions provided by Cayman Chemical. Briefly, the concentration of TXB_2 from the plasma sample binding to a limited amount of TXB_2 -specific antibodies in the well determines the intensity of the enzymatic reaction, which is assessed photo spectrometrically (410 nm).

For the calculation of the TXB_2 concentrations based on the absorbance readings, a standard curve was generated with eight standards of known concentrations. The ratio of standard bound/maximal bound (B/BO) for all standards was plotted against the TXB_2 concentration as a semi-logarithmic plot and a linear visual fit was carried out. With the equation generated from the standard curve and the B/BO ratio (sample bound/maximal bound) of each sample, the TXB_2 concentration of each plasma sample was calculated.

2.4 | Pharmacokinetic analysis

All pharmacokinetic parameters were calculated with Kinetica 5.0 (Thermo) using a noncompartmental model. The maximum plasma concentration (C_{\max}) and the time to maximum concentration (T_{\max}) were read directly of the concentration versus time plasma profile. The area under curve to the last quantifiable time point (AUC_{last}) was determined using the linear trapezoidal rule ($AUC_{\text{last}} = \sum_{i=1}^n 0.5 \times (C_i + C_{i+1}) \times \Delta t$). The total area under curve extrapolated to infinity (AUC_{tot}) was calculated as $AUC_{\text{tot}} = AUC_{\text{last}} + AUC_{\text{extra}} = AUC_{\text{last}} + C_{\text{last}}/\lambda$ with C_{last} being the computed last measured concentration and λ being the terminal elimination rate constant. The area under the moment curve from the time point zero to the last measured time point ($AUCM_{\text{last}}$) was calculated as $AUCM_{\text{last}} = \sum_{i=1}^n 0.5 \times (t_i \times C_i + t_{i+1} \times C_{i+1}) \times \Delta t$. The half-life of λ_z ($t_{1/2}$), clearance (Cl/F) and volume of distribution during terminal phase (V_z/F) and volume of distribution at steady-state (V_{ss}/F) and the mean residence time (MRT) were determined as $t_{1/2} = \ln(2)/\lambda$; $V_z = Cl/\lambda = \text{Dose}/(AUC_{\text{tot}} \times \lambda)$; $V_{ss} = (\text{Dose} \times \text{MRT})/AUC$, $Cl = \text{dose}/AUC_{\text{tot}}$ and $\text{MRT} = AUCM_{\text{tot}}/AUC_{\text{tot}}$.

3 | RESULTS

3.1 | Side effects

No adverse effects were observed at the site of injection after carprofen administration. All six rhinoceros ate within 12 hr after immobilization and displayed normal physiological behaviour. In four of six animals, a band like swelling was observed at the base of the ear in which the intravenous enrofloxacin was administered. The adverse

reaction appeared within the first 6 hr after drug administration and formed a painless oedema around the base of the ear. The swelling decreased in all affected individuals within 24 hr and disappeared or was significantly reduced towards the end of the study, after 72 hr.

3.2 | Pharmacokinetic analysis

All data are reported as geometric means (Gmean) and standard error of the mean ($\pm SEM$). After the single i.m. injection of 1 mg/kg, a carprofen concentration of $0.088 \pm 0.11 \mu\text{g/ml}$ was reached after 4.68 ± 0.54 min. The maximum carprofen concentration (C_{\max}) of $5.77 \pm 0.63 \mu\text{g/ml}$ was reached after 9.51 ± 3.53 hr. At the time of the last blood draw, after 73.07 ± 0.63 hr, carprofen plasma concentrations were still at $3.69 \pm 0.2 \mu\text{g/ml}$. Carprofen half-life of λ_z was estimated at 105.71 ± 15.67 hr and the mean residence time (MRT) in plasma at 155.01 ± 22.46 hr. The area under the curve extrapolated to infinity (AUC_{tot}) was $904.61 \pm 110.78 \mu\text{g ml}^{-1} \text{hr}^{-1}$. The mean clearance of carprofen was $0.0011 \pm 0.0001 \text{ L hr}^{-1} \text{kg}^{-1}$. The apparent volume of distribution at steady-state and at terminal phase was $0.17 \pm 0.01 \text{ L/kg}$. The pharmacokinetic parameters for all individuals are summarized in Table 2. The carprofen plasma concentration–time curve for each individual is shown in Figure 1, the average plasma concentration–time curve and the standard error of the mean for all individuals are presented in Figure 2.

3.3 | Pharmacodynamic analysis

TXB₂ plasma concentrations were determined by ELISA and were characterized by high inter-animal differences (Figure 1). In all

TABLE 2 Pharmacokinetic parameters of carprofen in six white rhinoceros after single intra muscular injection of 1 mg/kg

Parameter	Units	Animal						Mean	Gmean	SEM
		I	II	III	IV	V	VI			
λ	hr ⁻¹	0.005	0.008	0.004	0.010	0.008	0.006	0.007	0.007	0.00
$t_{1/2}$	hr	150.31	81.62	162.60	66.92	92.30	113.23	111.16	105.71	15.67
C_{\max}	$\mu\text{g/ml}$	4.27	8.56	6.49	5.40	6.19	4.64	5.93	5.77	0.63
T_{\max}	hr	23.67	6.32	6.35	22.02	5.88	6.03	11.71	9.51	3.53
AUC_{last}	$\mu\text{g ml}^{-1} \text{hr}^{-1}$	275.22	408.76	371.66	310.39	337.05	304.59	334.61	331.72	19.91
AUC_{tot}	$\mu\text{g ml}^{-1} \text{hr}^{-1}$	1,009.15	897.78	1,421.93	616.62	806.73	855.11	934.55	904.61	110.78
AUC_{extra}	$\mu\text{g ml}^{-1} \text{hr}^{-1}$	733.93	489.02	1,050.27	306.23	469.68	550.52	599.94	556.95	106.26
AUC_{extra}	%	72.73	54.47	73.86	49.66	58.22	64.38	62.22	61.57	4.02
$AUMC_{\text{last}}$	$\mu\text{g ml}^{-1} \text{hr}^{-2}$	9,650.94	13,019.90	12,886.80	10,462.30	11,649.80	11,006.90	11,446.00	11,380.00	547.01
Cl/F	$\text{L hr}^{-1} \text{kg}^{-1}$	0.0010	0.0011	0.0007	0.0016	0.0012	0.0012	0.0011	0.0011	0.00
V_z/F	L/kg	0.21	0.13	0.16	0.16	0.17	0.19	0.17	0.17	0.01
V_{ss}/F	L/kg	0.22	0.13	0.17	0.16	0.17	0.19	0.17	0.17	0.01
MRT	hr	219.63	117.71	235.58	100.95	135.58	166.44	162.65	155.01	22.46

λ , terminal elimination rate constant; $t_{1/2}$, half-life; C_{\max} , maximum plasma concentration; T_{\max} , time to maximum plasma concentration; AUC_{last} , area under the curve until the last time point; AUC_{tot} , area under the curve extrapolated to infinity; AUC_{extra} , area under the curve from the last quantifiable measurement to infinity; $AUMC_{\text{last}}$, area under the moment curve from $t = 0$ to the last measured time point; Cl/F , clearance corrected for bioavailability; V_z/F , apparent volume of distribution during the terminal phase corrected for bioavailability; V_{ss}/F , apparent volume of distribution in steady-state corrected for bioavailability; MRT, mean residence time.

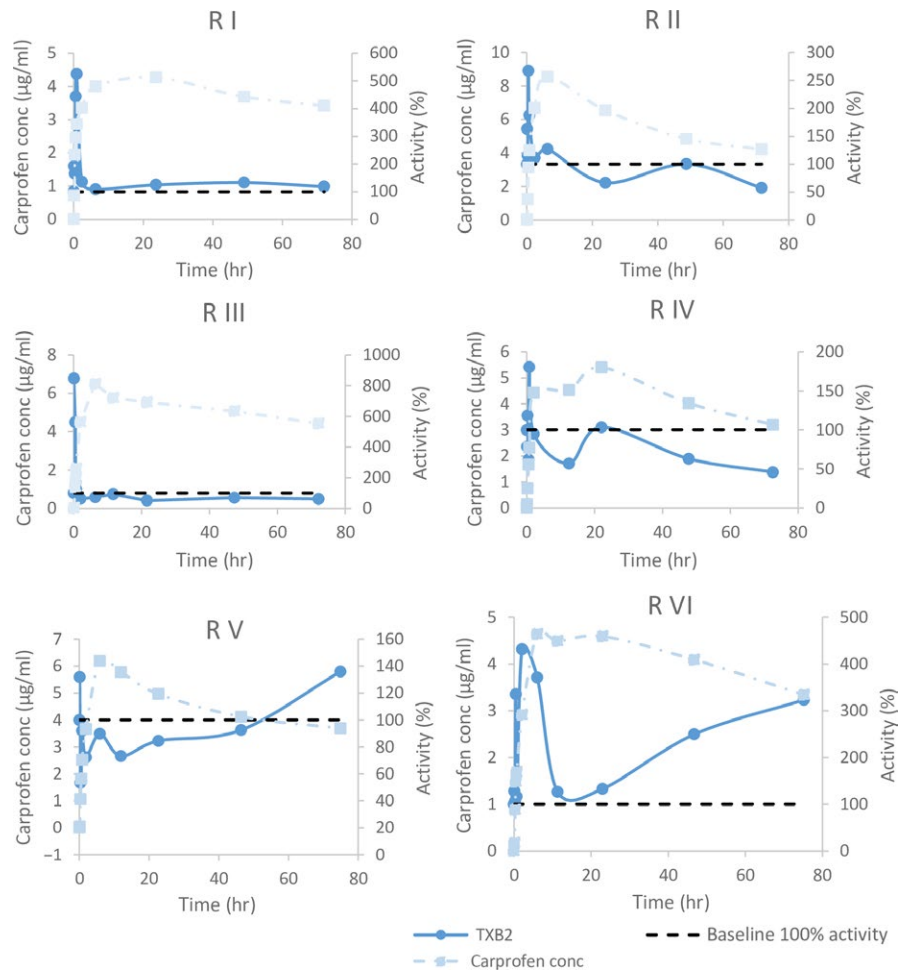


FIGURE 1 Time versus plasma drug concentration (primary axis) for carprofen (square) and % activity (as a percentage of the 0 hr sample, secondary axis) of TXB₂ (circle) versus time after a single intramuscular injection of 1 mg/kg carprofen in rhino I (R I) to rhino VI (R VI)

individuals, TXB₂ concentrations increased after carprofen administration and reached a peak concentration after 22.3 ± 18.46 min. The mean carprofen plasma concentration at the time of the peak was 0.6 ± 0.59 µg/ml. In rhino I, II and III the TXB₂ activity decreased rapidly after reaching the peak and remained around the initial TXB₂ concentration measured prior to the administration of carprofen. In rhino IV, the TXB₂ concentration continuously decreased after 22.02 hr until the end of the study (72.57 hr). In contrast, while the TXB₂ activity in rhino V and VI decreased after reaching the peak concentration, TXB₂ concentrations started to increase again after 11.88 hr (rhino V) and 11.32 hr (rhino VI) until the end of the study. The carprofen plasma concentration versus time profiles, as well as the TXB₂ versus time profiles for each individual and as the Gmean (\pm SEM) of all individuals, are depicted in Figures 1 and 2, respectively.

4 | DISCUSSION

Carprofen, a drug with antipyretic, anti-inflammatory and analgesic properties, is widely used in horses, cats and dogs for the management of skeletal, muscular and soft tissue injuries (Lees et al., 2002), as well as for the management of postsurgical pain.

Resting upon its favourable characteristics in domestic species and the positive feedback of wildlife veterinarians reporting long analgesic effects in rhinoceros suffering from lameness (personal communication, Dr. J. Marais, Saving the Survivors), it was deemed a potentially promising drug for the treatment of injured poaching victims. Based on the pharmacokinetic profile, we evaluated if carprofen administered i.m. at a dose of 1 mg/kg could be of value for the treatment of injuries in rhino. The dose of 1 mg/kg was selected based on the dose of 0.7 mg/kg in the horse and was chosen slightly higher to accommodate for potential interspecies differences and to be able to scale down rather than to scale up in case of a required dose adjustment. Carprofen was administered in conjunction with intravenous and oral enrofloxacin to mimic the field situation, where injured poaching victims generally require analgesic as well as antimicrobial treatment.

Carprofen was characterized by a fairly long absorptive phase with a T_{max} of 9.51 ± 3.53 hr (corresponding C_{max} of 5.77 ± 1.55 µg/ml). The absorptive phase compares favourably with the horse, which exhibits a comparable T_{max} of 10.6 ± 1.6 hr, albeit at a lower maximum concentration of 2.2 ± 0.4 µg/ml following a single dose of 0.7 mg carprofen/kg (McKellar et al., 1991). While we did not specifically evaluate the reason for the slow absorption of the drug from the site of injection, we believe that it may be attributed to

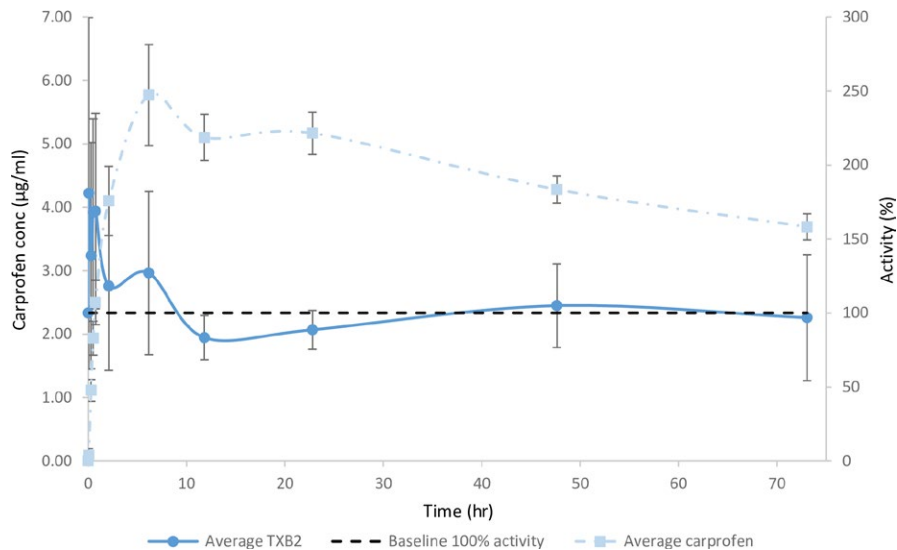


FIGURE 2 Time versus average plasma drug concentration (RI–RVI, primary axis) for carprofen (square) and % activity (as a percentage of the 0 hr sample, RI–RVI, secondary axis) of average TXB2 (circle) versus time

two possible causes. One reason may be the ability of the drug to precipitate or crystallize at the site of administration. This process has been evaluated in the horse, where an increase in creatinine kinase (CK) activity, a marker of muscle cell damage, was noted after i.m. carprofen injection (McKellar et al., 1991). From pharmacokinetic theory, it is known that localized irritation at the administration site can slow absorption (McKellar et al., 1991), which is used in formulation chemistry to create long-lasting effects for certain antibiotic formulations (Xia, Gyrd-Hansen, & Nielsen, 1983). The second reason for the slow absorption may be a drug interaction between etorphine and carprofen. From previous studies, vasoconstrictive effects have been reported following etorphine administration in both rhino and goats (Heard, Nichols, Buss, & Kollias, 1996; Heard, Olsen, & Stover, 1992). As such, the change in blood supply to the site of administration may be responsible for the delayed absorptive phase. Of the two causes, the latter is more likely, as no injection site reactions were visible clinically in any of the treated animals.

Another major finding for this study is the exceptionally long half-life of 105.71 ± 15.67 hr with large inter-animal variability. The half-life in the white rhino is substantially longer than the 29.4 ± 3 hr following a single i.m. dose at 0.7 mg/kg in the horse (McKellar et al., 1991); or the 30.7 ± 2.3 hr in the cow (Lohuis et al., 1991) and 26.1 ± 1.1 hr in sheep (0.7 mg/kg IV; Welsh et al., 1992). To our knowledge, the half-life in the white rhinoceros is the longest recorded half-life amongst all mammalian species. This in itself is not a very surprising finding, as previous studies we have undertaken with enrofloxacin have also shown the rhino to be a slow metabolizer, even when their size is taken into consideration. As a result, we speculate that the rhino is metabolically constrained.

Along with the metabolic restrictions, the slower clearance and prolonged half-life of carprofen may result from a drug–drug interaction. Enrofloxacin, a fluoroquinolone, has been reported to interact with the pharmacokinetics of other drugs such as the

methylxanthine theophylline (Intorre, Mengozzi, Maccheroni, Bertini, & Soldani, 1995) and NSAIDs on several occasions (Abo-El-Sooud & Al-Anati, 2011; Ogino, Mizuno, Ogata, & Takahashi, 2005; Rahal, Kumar, Ahmad, & Malik, 2008). The reason for the interaction of enrofloxacin with the pharmacokinetics of certain drugs is thought to be linked to the inhibition of particular drug metabolizing cytochrome P450 (CYP) enzymes, such as CYP1A1 and CYP1A2 (Intorre et al., 1995; Rahal et al., 2008; Regmi, Abd El-Aty, Kuroha, Nakamura, & Shimoda, 2005; Sasaki & Shimoda, 2015; Vancutsem & Babish, 1996). While enrofloxacin seems to increase the half-life of elimination of theophylline, the effect on the metabolism of NSAIDs seems to be less consistent (Intorre et al., 1995). Flunixin in conjunction with enrofloxacin (5 mg/kg s.c.) in dogs leads to a significant increase in the elimination half-life by 29% (Ogino et al., 2005); while the administration of diclofenac with enrofloxacin in sheep results in a slowed absorption and a prolonged elimination half-life (57%; Rahal et al., 2008). In contrast, in calves, enrofloxacin induces a 27% decrease in the half-life of elimination of flunixin (Abo-El-Sooud & Al-Anati, 2011). In horses, however, the concomitant administration of enrofloxacin at a dose of 5 mg/kg with firocoxib does not change the pharmacokinetics of firocoxib (Cox et al., 2012). With specific information on interaction of fluoroquinolones and carprofen currently unavailable, it is difficult to establish if the long half-life of carprofen results from an interaction or a general metabolic constraint in the rhinoceros. Nonetheless, based on the extremely long half-life of carprofen in the rhino, enzyme inhibition alone would probably not explain the latter. To definitely answer this question, the pharmacokinetics of carprofen would need to be established in a single drug study.

To evaluate the duration of benefits of carprofen, we assessed its effects on TXB₂ plasma concentrations. In general, the NSAIDs are known to, in part function through the inhibition of the cyclooxygenase 1 and 2 (COX-1 and 2) leading to a reduced production of eicosanoids and as a result, inhibiting inflammatory reactions, pain

and hypersensitivity (Moses & Bertone, 2002). The constitutively expressed COX-1 plays a less significant role in cases of tissue injury and inflammation than COX-2, however, increases by 2- to 3-fold after tissue injuries and plays a role in pain transmission (Brooks et al., 1999). In contrast, COX-2 enzymes are induced and upregulated during inflammatory processes and in case of tissue damage. Their concentrations can raise 20-fold above the baseline values (Brooks et al., 1999; Lee et al., 1992).

To assess the degree of COX-1 inhibition of NSAIDs, TXB₂ is commonly used as a surrogate marker (Lees et al., 2004). We were expecting TXB₂ to decline as described in species such as the horse and the cat (Lees, McKellar, May, & Ludwig, 1994; Taylor et al., 1996). In horses, studies demonstrated a moderate inhibition of TXB₂ at low doses of carprofen (0.7 mg/kg) and complete inhibition at high carprofen doses (4 mg/kg; Lees et al., 1994, 2002). However, as with field-type trials, we experienced an unexpected adverse reaction for the concurrently administered enrofloxacin. The enrofloxacin administration caused a visible, localized adverse reaction in four of six rhinoceros (rhino I, III, IV, VI). It manifested in the form of a band like swelling at the base of the ear in which enrofloxacin was injected. This was evident in the plasma, as a rapid increase in TXB₂ concentrations with the peak of activity after 22.3 ± 18.46 min (TXB₂), which was followed by a rapid decline. Using this unexpected reaction, we are able to provide an indication of the expected duration of effect of carprofen. Firstly, the early decline of the TXB₂ concentration after 22.3 ± 18.46 min indicates a rapid onset of effect of carprofen, which is consistent with the results from a study by Borer, Peel, Seewald, Schawalder, and Spreng (2003). Using an acute synovitis model, Borer et al. (2003) assessed the analgesic and anti-inflammatory properties of carprofen in dogs and recorded an analgesic effect after 2.6 hr.

Besides assessing the plasma drug concentration, an important consideration when looking at the duration of effect of the NSAIDs is the duration of enzyme inhibition. Studies by Lees et al. (1994) in horses have shown no identifiable relationship between the carprofen plasma concentration and the effect on the COX enzymes. This has led to the concept of a pharmacodynamic half-life, which describes the relation between the effect (E) of a drug and the time (t). A mathematical relationship can be established between the pharmacokinetic and pharmacodynamic half-life and shows that for high drug concentrations, the pharmacodynamic half-life is considerably longer than the pharmacokinetic half-life, leading to a longer lasting drug effect (Keller, Czock, Zellner, & Giehl, 1998). As an example, the concept of the pharmacodynamic half-life could explain why carprofen in dogs exhibits a relatively short half-life of 7.99 ± 2.89 hr, while its postsurgical analgesic effect lasts for about 24 hr (McKellar et al., 1990; Shih, Robertson, Isaza, Pablo, & Davies, 2008). Based on the effective concentration and the secondary peak of TXB₂ production in rhino V and VI after 48 hr, we believe that the drug would be effective for a minimum of 48 hr after single carprofen administration. Nonetheless, these results have to be assessed with caution as the interpretation of the data is complicated by the lack

of obvious correlation between the degree of TXB₂ inhibition and carprofen plasma concentrations, as described by Lees et al. (1994) in the horse.

While we are confident of the anti-inflammatory effect of carprofen in white rhino, we cannot conclusively show that it will be effective as an analgesic. To try to ascertain the value of the pharmacokinetic parameters, the concentrations achieved were compared to results from Schatzmann et al. (1990) who assessed the peripheral pain inhibition of carprofen in horses. Using the heating element model, the study demonstrated that 0.7 mg carprofen/mg successfully inhibited pain for roughly 24 hr. The analgesic effect of carprofen was correlated to a plasma concentration of at least 1.5 µg/ml. When comparing the effective carprofen plasma concentration of at least 1.5 µg/ml in the horse to the rhino pharmacokinetic profile, the plasma concentration of 1.5 µg/ml was achieved as early as 31 ± 0.98 min after carprofen administration and was maintained beyond the 72-hr monitoring period, with the carprofen plasma concentration still above 3.68 ± 0.2 µg/ml. While we suggest that actual pain monitoring needs to be conducted in rhino treated with carprofen, we would expect the drug to be effective in most animals for a minimum of 48 hr.

5 | CONCLUSION

Wildlife veterinarians have documented good analgesic effects when using carprofen in injured white rhinoceros in the field (personal communication, Dr. Johan Marais, Saving the Survivors). To scientifically evaluate carprofen for a safe and efficient application in white rhinoceros, this first of its kind study investigated the pharmacokinetic and pharmacodynamic properties after a single dose injection. We were able to demonstrate that the pharmacokinetic profile of carprofen in rhinoceros differed significantly from that of the horse and any other species. Carprofen was characterized by a remarkable long half-life of $\lambda_{1/2}$, which is longer than the reported half-lives in any mammalian species. Based on the evaluation of the surrogate marker TXB₂, the drug also appeared to be anti-inflammatory for a minimum of 48 hr after administration in most animals, making it a promising drug to consider and further evaluate for the management of pain and inflammation in white rhinoceros.

ACKNOWLEDGMENTS

This study was made possible through the help of "The Rhino Orphanage," it's founder Mr. Arrie van Deventer who allowed us to carry out the field work and data collection at "The Rhino Orphanage" and the staff, in particular Laura Ellison and Jamie Traynor, who helped with the implementation of the field work and the data collection. Furthermore, we would like to thank Dr. Robin Gieling, Aminisha Somers and Klaudia Pruckowska for their assistance during the field work. We thank Dr Pierre Bester for the chemical immobilization and the monitoring of the rhinoceros during the trial. Furthermore, a sincere thank you goes to the German

Academic Exchange Service (DAAD), the University of Pretoria, the South African Veterinary Association (SAVA) and Bayer Animal Health for their financial and material support.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interests.

AUTHOR CONTRIBUTION

All authors have read and approved the final manuscript. ML: conceptualization, formal analysis, funding, investigation, methodology, project administration, visualization, writing—original draft preparation and validation. RK: formal analysis and validation. JM: conceptualization, funding, methodology and supervision. MH: conceptualization, methodology, supervision and investigation. VN: conceptualization, funding, methodology, visualization, validation, formal analysis and supervision.

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How to cite this article: Leiberich M, Krebber R, Hewetson M, Marais J, Naidoo V. A study of the pharmacokinetics and thromboxane inhibitory activity of a single intramuscular dose of carprofen as a means to establish its potential use as an analgesic drug in white rhinoceros. *J vet Pharmacol Therap.* 2018;00:1–9. <https://doi.org/10.1111/jvp.12508>