Pharmacokinetics of an intravenous and oral dose of enrofloxacin in white rhinoceros (*Ceratotherium simum)*

Enrofloxacin in white rhinoceros

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1. Abstract

South Africa currently loses over 1000 white rhinoceros (*Ceratotherium simum*) each year to poaching incidents, and numbers of severely injured victims found alive have increased dramatically. However, little is known about the antimicrobial treatment of wounds in rhinoceros. This study explores the applicability of enrofloxacin for rhinoceros through the use of pharmacokinetic-pharmacodynamic modelling. The pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin were evaluated in five white rhinoceros after intravenous (IV) and after successive IV and oral administration of 12.5 mg/kg enrofloxacin. After IV administration, the half-life, area under the curve (AUC_{tot}), clearance and the volume of distribution were 12.41 ± 2.62 hours, 64.5 ± 14.44 µg/ml*h, 0.19 ± 0.04 L/h*kg and 2.09 ± 0.48 L/kg, respectively. Ciprofloxacin reached 26.42 ± 0.05 % of the enrofloxacin plasma concentration. After combined IV and oral enrofloxacin administration oral bioavailability was 33.30 ± 38.33%. After IV enrofloxacin administration, the efficacy marker AUC_{24}: MIC exceeded the recommended ratio of 125 against bacteria with an MIC of 0.5 µg/mL. Subsequent intravenous and oral enrofloxacin administration resulted in a low Cmax: MIC ratio of 3.1. The results suggest that intravenous administration of injectable enrofloxacin could be a useful drug with bactericidal properties in rhinoceros. However, the maintenance of the drug plasma concentration at a bactericidal level through additional per os administration of 10 % oral solution of enrofloxacin indicated for the use in chickens, turkeys and rabbits does not seem feasible.

Keywords: Enrofloxacin, fluoroquinolone, antimicrobial drug, white rhinoceros, poaching

2. Introduction

The white rhinoceros (*Ceratotherium simum*), one of Africa's iconic species, is in danger of extinction due to unscrupulous poaching. The illegal killing is driven by the demand for rhino horn used in traditional Chinese medicine, for ceremonial purposes and as a status symbol mainly in Asian countries (Challender & MacMillan, 2014). Figures published in 2018 report 1215 deaths in 2014, up from 1004 and 668 in 2013 and 2012, respectively. In 2015 and 2016 another 1175 and 1054 rhinos were killed for their horn (Poaching statistics, 2018). Furthermore, in addition to the dramatic increase in killed rhinoceros, the number of rhinos escaping immediate death has been on the rise, with an estimated 200 animals needing veterinary assistance per year (J. Marais, personal communication, 2016). Injuries seen in these animals included limb wounds caused by snares such as abrasions, tearing of the skin, swelling and muscle damage. Deep gun-shot wounds in the limbs, the head or the torso are common with resultant blood loss, anaemia, hypovolemia, fractures, septic joints and soft tissue secondary infections. Extensive facial wounds with resultant exposed frontal and nasal sinuses after the brutal removal of the horns are found more and more often (Cooper & Cooper, 2013).

Injured animals require immediate veterinary treatment, which involves stabilizing the patient, haemostatic measures, various diagnostic measures such as radiography and typically wound management including surgical lavage and wound dressing. Analgesic and antimicrobial support is vitally important in all these rhinos. Unfortunately, despite the necessity for proper therapeutic measures, pharmaceutical agents active against infection and pain are yet to be evaluated. As a result, current therapies are extrapolated from other veterinary species. Species-specific knowledge is needed urgently; however, the research of drug pharmacokinetics and pharmacodynamics in non-domesticated species is challenging due to the inability to safely get into close contact, the difficulties with frequent re-administration and the need for large volumes of drug.

The focus of this study was to optimize the antimicrobial treatment of rhinos by having at least one scientifically evaluated antimicrobial drug available. Initial criteria set for this optimal agent were as follows: the ideal drug should be broad spectrum to allow for treatment in the field where culture and antibiograms are not always feasible and should have a prolonged mean residence time to prevent frequent re-administration. It should be commercially available as a sufficiently concentrated formulation in order to reduce the

dosing volume required (reducing the number of injections per administration). Furthermore, it should be available as an oral, water soluble medication so that treatment can be continued in the drinking water or feed while the animal recovers in an enclosure with minimal human contact (minimise stress and injury from requiring re-immobilisation of the already compromised animal for re-administration).

In the course of the drug selection process, we also screened a database of previously evaluated white rhinoceros bacterial culture results from the bacteriology laboratory of the Department of Tropical Diseases, University of Pretoria obtained between 2008 and beginning of 2015. Of the 33 recorded cases (excluding faecal samples), 15 samples underwent antimicrobial susceptibility testing and revealed that enrofloxacin was one of the antimicrobials with a high susceptibility rate (60%). Based on this criterion and the promising pharmacokinetic characteristics, we selected enrofloxacin, a second-generation fluroquinolone for further study. Enrofloxacin, the first fluoroquinolone developed for veterinary purposes, is a broad spectrum antimicrobial, particularly effective against gram-negative bacteria, and most importantly exhibits a rapid bactericidal, concentration dependent effect, which would allow a once daily treatment. Another major advantage is that the product is already available as an oral and parenteral formulation (Lode, Borner, & Koeppe, 1998; Lopez-Cadenas et al., 2013) at a relatively high concentration of 100 mg/ml, which could allow the stress-free oral administration of the drug in the drinking water or feed.

3. Materials and Methods

3.1. Experimental Design

The study was divided into two phases, and was approved by the Animal Ethics Committee of the University of Pretoria (permit number: V074-15). For the first phase, five rhinoceros were administered a single intravenous dose of enrofloxacin at 12.5 mg/kg (Baytril, Injectable, Bayer Animal Health, 100 mg/ml) with an i.m. injection of 1 mg/kg of racemic carprofen (Rimadyl Injection, 50 mg/kg Zoetis) as concurrent anti-inflammatory treatment (results to be presented in a different article). The second phase began after a washout period of eight weeks. All animals were again treated with a single intravenous dose of enrofloxacin at 12.5 mg/kg (Baytril, Injectable, Bayer Animal Health, 100 mg/ml) and a single intramuscular dose of carprofen at 1 mg/kg (Rimadyl, Zoetis, 50 mg/ml). The parenteral drug administration was followed by *per os* enrofloxacin at 12.5 mg/kg (Baytril, Bayer Animal Health, 10 % oral solution, indicated for the use in chickens, turkeys and rabbits). The oral solution was administered in the feed. The liquid enrofloxacin was diluted with an equal volume of water

and poured over about two scoops of pellets. After absorption of the enrofloxacin-water mixture by the pellets, the medicated pellets were mixed with two scoops of non-medicated pellets and two handfuls of lucerne (*Medicago sativa*). To mask the bitter taste, a small amount of molasses was added and the ingredients were blended thoroughly until evenly mixed. The total amount of food was weighed before being fed to the animals in order to be able to calculate the exact amount of ingested feed. The results from the first phase have been partially presented in a publication on the allometric scaling of enrofloxacin in the white rhinoceros (submitted to PlosOne).

3.2. Animals

Five habituated white rhinoceros (one female, four males) from the 'The Rhino Orphanage' in South Africa were used for the study (S 1 table). The minimum age was 13 months and the average weight of the animals was 623 kg and 670 kg in the first and second phase, respectively. The rhinoceros graze in groups in large enclosures during daytime and sleep in large enclosed paddocks or the attached night-rooms. Besides the grazing, the animals receive additional feed consisting of teff (*Eragrostis teff*), lucerne and pellets twice daily and water *ad libitum*. Rhino I and rhino II also received a milk feed of one litre, twice daily during the first phase of the trial. For the period of each trial, the animals were kept in a boma in groups of two to three animals with free access to water and to their daily feeds. Prior to the start of the study, the animals were trained (positive operant conditioning training) to tolerate the touching of their ears for the sample collection through the catheter. To reduce stress during the blood collection phase of the study, animals were administered a single dose of the long acting tranquiliser zuclopenthixol acetate (Clopixol-Acuphase, 50 mg/ml, Lundbeck) at 50 mg/animal intramuscular (Kock & Burroughs, 2012).

3.3. Experimental procedures

3.3.1. Blood Sampling

The plasma concentration of enrofloxacin and its active metabolite ciprofloxacin were evaluated over a period of 72 hours. Blood samples were collected prior to administration and around 5, 15, 30, 45 minutes and 2, 6, 12, 24, 48, 72 hours after administration of enrofloxacin. Due to difficulties in approaching the animals for direct venepuncture, the rhinoceros had to be sedated for the placement of a catheter. After the 12-hour bleed, blood was collected under sedation directly from the cephalic vein. In all cases, the immobilization process closely followed that of field management of rhino in South Africa.

3.4. Analysis of the Enrofloxacin and Ciprofloxacin Plasma Concentrations via Online - Solid Phase Extraction/ Tandem Mass Spectrometry

All blood samples were placed on ice immediately after collection and centrifuged at 3000 rpm for 15 minutes within 4 hours of collection. Plasma samples were stored at -20°C for a maximum of 8 days at the study site prior to being transferred into the -80°C freezers of the University of Pretoria. For evaluation, samples were shipped to Germany on dry ice (World Courier) for analysis by Bayer Animal Health (CITES export permit number: 152722) and analysed by a previously validated method, namely the online – solid phase extraction/ tandem mass spectrometry (online-SPE-MS/MS). The measurement conditions in general have been described by Krebber et al. (2009), with the only modification being the replacement of trifluoroacetic acid by heptafluorobutyric acid as described by Bousova et al (2013).

3.5. Assessment of the Pharmacokinetics of Enrofloxacin and Ciprofloxacin

The plasma concentration of enrofloxacin and its active metabolite ciprofloxacin were determined for each individual at the different points of time. All pharmacokinetic calculations were undertaken in Kinetica 5.0 (Thermo). The following pivotal non-compartmental parameters were calculated for enrofloxacin and ciprofloxacin: 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 ($AUC_{last} = \sum_{i=1}^{n} 0{,}5 * ((C_i + C_{i+1}) * \Delta t)$). The total area under curve (extrapolated to infinity) (AUC_{tot}) was calculated as follows: $AUC_{tot} = AUC_{last} + AUC_{extra} = AUC_{last} + C_{Last}/\lambda$ 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 ($AUCM_{last}$) was calculated as $AUMC_{last} = \sum_{i=1}^{n} 0{,}5 * (t_i * C_i + t_{i+1} * C_{i+1}) * \Delta t$. The half-life ($t_{1/2}$), clearance (Cl) and volume of distribution during terminal phase (V_z) and volume of distribution at steady state (V_{ss}) and the mean residence time (MRT) were determined as $t_{1/2} = \ln(2)/\lambda$; $V_z = Cl/\lambda = Dose/(AUC*\lambda)$; $V_{ss} = (Dose*MRT)/AUC$, $Cl = dose/AUC_{tot}$ and $MRT = AUMC_{tot}/AUC_{tot}$. The oral bioavailability of enrofloxacin was calculated as $F = (AUC_{PO}/Dose_{PO})/(AUC_{IV}/Dose_{IV})$, where the $Dose_{PO}$ was the dose of the orally administered enrofloxacin and AUC_{IV} and $Dose_{IV}$ were the AUC_{tot} and the dose of the intravenously administered enrofloxacin. The AUC_{PO} was estimated as the AUC_{tot} of the first phase subtracted from the AUC_{tot} of the second phase.

3.6. Assessment of the Pharmacodynamics of Enrofloxacin and Ciprofloxacin

In order to predict the therapeutic use of enrofloxacin, the surrogate markers AUC_{24}: MIC and C_{max}:AUC after IV administration were evaluated. With enrofloxacin being partially transformed to the active metabolite ciprofloxacin, the total AUC_{24} was determined as AUC_{24enro} + $AUC_{24cipro}$. The MIC value of 0.5 used for the calculation of the ratio represents the susceptibility breakpoint for enrofloxacin published by the CLSI (CLSI, 2015). Furthermore, the change in slope of the semilogarithmic plot of the enrofloxacin concentration was used as a brief indicator for the pseudo Cmax of the addititve curve after subsequent intravenous and oral enrofloxacin administration.

4. Results

4.1. Side Effects

No adverse effects were observed during the first phase of the study. During the second phase of the study, four out of five rhinos developed a band like swelling at the base of the ear in which enrofloxacin was injected. The swelling appeared within the first six hours after the injection through the auricular catheter and consisted of a painless oedema around the base of the ear. The swelling decreased in all affected individuals within 24 hours and either disappeared or was significantly reduced towards the end of the study, after 72 hours. Apart from the swelling at the base of the ear, the rhinoceros showed no further side effects and did not seem affected by the reaction. All rhino ate within 12 hours after immobilisation and exhibited their normal physiological behaviour. One rhinoceros developed a thrombophlebitis in the auricular vein where the long stay catheter was placed. It was discovered one month after the end of the study. It was assessed by the local veterinarian; it was kept clean and healed without further complications.

4.2. Blood Sampling

Despite every effort to facilitate blood collection at the scheduled intervals, this was not accurately possible due to the challenges of working with wild animals. On average, the blood sampling during the first trial took place prior and 8.8, 23.2, 37.4, 52.6 minutes and 2.11, 6.37, 12.33, 24.94, 48.30 und 71.45 hours after the injection of enrofloxacin. For the second trial, the five animals received an enrofloxacin treatment as in the first trial (12.5 mg enrofloxacin/kg body weight i.v.) followed by an oral once off enrofloxacin medication in the feed of 12.5 mg enrofloxacin/kg body weight. The treated food was ingested on average 10.06 ± 1.74h after intravenous enrofloxacin administration. All individuals ingested the full portion

of food with the complete amount of enrofloxacin, indicating that the method of dosing was acceptable. The blood sampling took place before and 7.6, 21.2, 33.4, 48.4 minutes and 2.2, 6.28, 11.92, 22.89, 47.95 and 72.76 hours after enrofloxacin injection. The actual times of collection were used in the subsequent pharmacokinetic analysis.

4.3. Pharmacokinetics of enrofloxacin and ciprofloxacin after intravenous enrofloxacin administration (Phase I)

All data is reported as geometric mean (Gmean) and standard deviation (± SD) for both phases. An enrofloxacin plasma concentration of 13.9 ± 3.70 µg/ml was recorded at the first sampling point post enrofloxacin injection after 8.8 ± 2.4 minutes. Due to challenges during the sample collection, at the last blood sampling point 71.45 ± 0.8 hours post enrofloxacin injection, only four rhinoceros could be sampled. Of the four rhinoceros, one exhibited an enrofloxacin concentration below the limit of quantification (LOQ < 0.02 µg/ml), while the other three rhinoceros exhibited an average enrofloxacin plasma concentration of 0.054 ± 0.02. Enrofloxacin was characterised by a long half-life of elimination ($t_{1/2}$) of 12.41 ± 2.62 hours. The area under the curve extrapolated to infinity (AUC_{tot}) was 64.5 ± 14.44 µg/ml*h. The clearance (Cl) was slow with a value of 0.19 ± 0.04 L/h*kg. The volume of distribution in steady state (V_{ss}) was 2.09 ± 0.48 L/kg. The residence time (MRT) in the plasma was 10.8 ± 1.67 hours. The formation of the active metabolite ciprofloxacin began rapidly. At the first sampling point post enrofloxacin injection, ciprofloxacin concentration was 0.15 ± 0.05 µg/ml and reached its maximum (C_{max}) of 0.92 ± 0.11 µg/ml after 2.1 ± 0.18 hours. At the last blood sampling point after 71.45 hours, ciprofloxacin concentrations of three rhinoceros were below the limit of quantification while one rhinoceros showed a quantifiable concentration of 0.03 µg/ml. The half-life ($t_{1/2}$) was 11.62 ± 1.28 hours. The AUC_{tot} was 17.04 ± 3.84 µg/ml*h. The plasma ciprofloxacin concentration reached 26.42 ± 0.05 % of the plasma enrofloxacin concentration. The results of the pharmacokinetic analysis of enrofloxacin and its active metabolite ciprofloxacin after a single intravenous enrofloxacin injection (12.5 mg/kg) are summarized in Table 1 and Table 2. The mean plasma concentration versus time curve of enrofloxacin and its active metabolite ciprofloxacin is depicted in Fig. 1 and the individual plasma concentration versus time profiles are depicted in S 2 fig.

Table 1: Pharmacokinetic parameters of enrofloxacin for each rhinoceros after intravenous administration (12.5 mg/kg) in phase I

Parameter	Units	Animal					Mean	Gmean	SD
		I	II	III	IV	VI			
λ	h^{-1}	0.05	0.07	0.04	0.07	0.05	0.06	0.05	0.01
t1/2	H	14.22	10.27	15.9	9.71	13.04	12.63	12.41	2.62
C_{max}	µg/ml	14.81	10.30	11.51	19.81	14.90	14.27	13.90	3.70
T_{max}	H	0.1	0.15	0.2	0.17	0.12	0.148	0.14	0.04
AUC_{last}	µg/ml*h	57.95	53.94	87.87	68.30	54.60	64.53	63.40	14.26
AUC_{tot}	µg/ml*h	58.61	54.36	89.65	68.48	57.10	65.64	64.50	14.44
AUC_{extra}	µg/ml*h	0.64	0.42	1.78	0.18	2.50	1.10	0.73	0.99
AUC_{extra}	%	1.76	1.25	3.17	0.42	7.02	2.72	1.83	2.60
$AUMC_{last}$	µg/ml*$(h)^2$	577.40	583.01	979.13	547.18	478.99	633.14	612.76	197.79
Clearance	L/h*kg	0.21	0.23	0.14	0.18	0.22	0.20	0.19	0.04
Vz	L/kg	4.38	3.41	3.2	2.56	4.12	3.53	3.47	0.73
Vss	L/kg	2.32	2.62	1.78	1.5	2.47	2.14	2.09	0.48
MRT	H	10.88	11.4	12.78	8.21	11.29	10.91	10.80	1.67

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; AUCextra, area under the curve from the last quantifiable measurement to infinity; $AUCM_{last}$, area under the moment curve from t =0 to the last measured time point; Cl, clearance; Vz, apparent volume of distribution during the terminal phase; Vss, apparent volume of distribution in steady state; MRT, mean residence time

Table 2: Pharmacokinetic parameters of ciprofloxacin after intravenous enrofloxacin administration (12.5 mg/kg) for each rhinoceros in phase I

Parameter	Units	Animal					Mean	GMean	SD
		I	II	III	IV	VI			
λ	h^{-1}	0.06	0.06	0.05	0.07	0.06	0.06	0.06	0.01
t1/2	h	12.55	10.77	13.48	10.56	11.01	11.67	11.62	1.28
C_{max}	µg/ml	0.99	0.87	1.08	0.94	0.78	0.93	0.92	0.11
T_{max}	h	1.98	1.82	2.27	2.22	2.27	2.11	2.10	0.18
AUC_{last}	µg/ml*h	17.03	17.82	21.81	17.66	11.02	17.07	16.67	3.87
AUC_{tot}	µg/ml*h	17.25	17.99	22.38	17.82	11.60	17.41	17.04	3.84
AUC_{extra}	µg/ml*h	0.21	0.18	0.57	0.17	0.58	0.34	0.29	0.22
AUC_{extra}	%	1.99	1.58	4.09	1.49	8.07	3.44	2.74	2.79
$AUMC_{last}$	µg/ml*$(h)^2$	228.25	261.84	369.50	241.37	138.43	247.88	236.37	82.75
MRT	h	14.36	15.41	18.82	14.34	15.12	15.61	15.53	1.85

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; $AUCM_{last}$, area under the moment curve from t =0 to the last measured time point; MRT, mean residence time

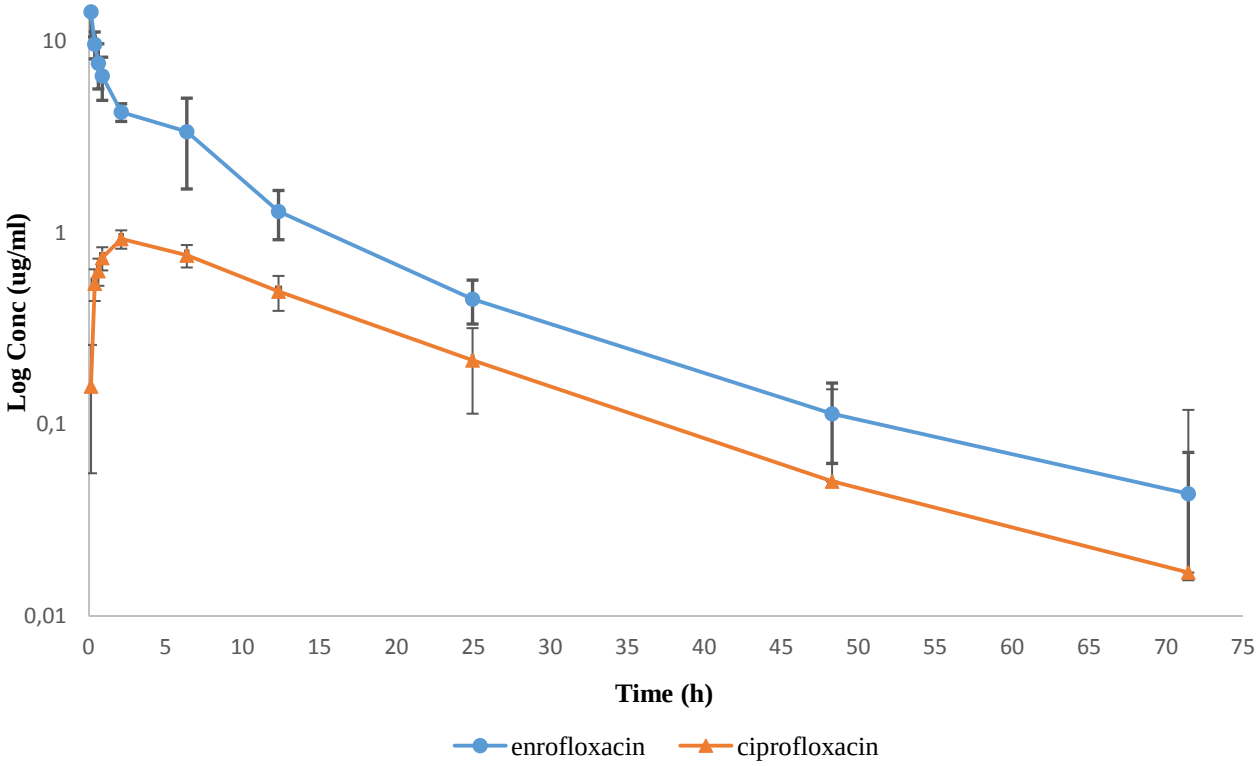

Fig. 1: average plasma concentration versus time profile of all 5 rhinoceros after IV administration of enrofloxacin (circle) at 12.5 mg/kg and its ciprofloxacin (triangle) metabolite

4.4. Pharmacokinetics of enrofloxacin and ciprofloxacin after intravenous and oral enrofloxacin administration (Phase II)

In the second phase of the study, enrofloxacin was administered intravenously and after an average of 10.16 ± 1.74 hours, a second dose of enrofloxacin (12.5 mg/kg) was given to the animals orally. Enrofloxacin plasma concentration 7.8 ± 1.8 minutes post enrofloxacin administration was 19.64 ± 8.05 µg/ml. At the last sampling point after 72.76 ± 1.41 hours, the average plasma concentration was 0.07 ± 0.02 µg/ml and all animals exhibited an enrofloxacin plasma concentration above the limit of quantification (0.02 µg/ml). The half-life ($t_{1/2}$) of enrofloxacin was 11.5 ± 0.84 hours and the MRT was 15.15 ± 1.5 hours. The AUC_{tot} was 92.38 ± 12.14 µg/ml*h. The mean Cl was 0.14 ± 0.02 L/h*kg and the apparent V_{ss} was 2.05 ± 0.14 L/kg. The estimated fraction of absorption of enrofloxacin was 33.3 ± 38.34 %.

At the first sampling point post enrofloxacin injection after 7.8 ± 1.8 minutes, ciprofloxacin concentrations reached in average 0.13 ± 0.03 µg/ml. The maximum ciprofloxacin concentration (C_{max}) of 0.71 ± 0.11 µg/ml was reached after 2.2 ± 2.1 hours. At the last sampling point (72.76 ± 1.41 hours), ciprofloxacin concentrations in one rhinoceros were below the limit of quantification (0.02 µg/ml), while the remaining four animals had an average concentration of 0.034 ± 0.01 µg/ml. The $t_{1/2}$ was 14.89 ± 1.32 hours. The MRT of ciprofloxacin was 21.69 ± 1.19 hours and the AUC_{tot} was 20.27 ± 3.42 µg/ml*h. Ciprofloxacin plasma concentrations reached 21.95 % of the plasma concentration of the parent drug as compared to 26.42 % in the first phase. The results of the kinetic analysis are summarized in Table 3 and Table 4. The mean plasma concentration versus time curve of enrofloxacin and its active metabolite ciprofloxacin is depicted in Fig. 2 and the enrofloxacin and ciprofloxacin concentration versus time curves for each individual are presented in S 3 fig.

Table 3: Pharmacokinetic parameters of enrofloxacin after intravenous and oral enrofloxacin administration for each rhinoceros in phase II (12.5 mg/kg)

Parameter	Unit	Animal					Mean	GMean	SD
		I	II	III	IV	VI			
λ	h^{-1}	0.067	0.063	0.057	0.058	0.057	0.060	0.060	0.005
t1/2	h	10.31	10.98	12.26	11.95	12.11	11.52	11.50	0.84
C_{max}	µg/ml	18.50	23.70	33.30	13.60	14.70	20.76	19.64	8.05
T_{max}	h	0.13	0.10	0.10	0.13	0.17	0.13	0.12	0.03
AUC_{last}	µg/ml*h	87.49	110.30	95.70	79.12	86.72	91.86	91.28	11.86
AUC_{tot}	µg/ml*h	88.24	111.77	97.24	80.07	87.62	92.99	92.38	12.14
AUC_{extra}	µg/ml*h	0.75	1.47	1.54	0.96	0.90	1.13	1.08	0.36
AUC_{extra}	%	1.37	2.11	2.54	1.91	1.65	1.92	1.88	0.45
$AUMC_{last}$	µg/ml*(h)²	1190.58	1770.24	1375.74	971.15	1321.40	1325.82	1300.56	293.30
Cl	L/h*kg	0.14	0.11	0.13	0.16	0.14	0.14	0.14	0.02
Vz	L/kg	2.11	1.77	2.27	2.69	2.49	2.27	2.24	0.35
Vss	L/kg	2.02	1.90	2.00	2.06	2.29	2.05	2.05	0.14
MRT	h	14.24	16.99	15.57	13.20	16.03	15.21	15.15	1.50

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; $AUCM_{last}$, area under the moment curve from t =0 to the last measured time point; MRT, mean residence time

Table 4: Pharmacokinetic parameters of ciprofloxacin after intravenous and oral enrofloxacin administration (12.5 mg/kg) for each rhinoceros in phase II (12.5 mg/kg)

Parameter	Units	Animal					Mean	Gmean	SD
		I	II	III	IV	VI			
1	h^{-1}	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.00
t1/2	h	14.90	15.29	16.54	15.09	12.88	14.94	14.89	1.32
C_{max}	µg/ml	0.63	0.81	0.83	0.72	0.58	0.71	0.71	0.11
T_{max}	h	0.90	6.33	2.00	2.10	2.13	2.69	2.20	2.10
AUC_{last}	µg/ml*h	18.87	24.58	20.33	19.29	15.85	19.78	19.59	3.16
AUC_{tot}	µg/ml*h	19.49	25.56	21.36	19.97	16.12	20.50	20.27	3.42
AUC_{extra}	µg/ml*h	0.62	0.98	1.03	0.68	0.27	0.72	0.65	0.31
AUC_{extra}	%	5.07	6.14	7.74	5.45	2.70	5.42	5.13	1.83
$AUMC_{last}$	µg/ml*$(h)^2$	353.54	486.28	395.22	367.11	299.19	380.27	375.48	68.78
MRT	h	21.10	22.62	23.13	21.59	20.14	21.72	21.69	1.19

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; $AUCM_{last}$, area under the moment curve from t =0 to the last measured time point; MRT, mean residence time

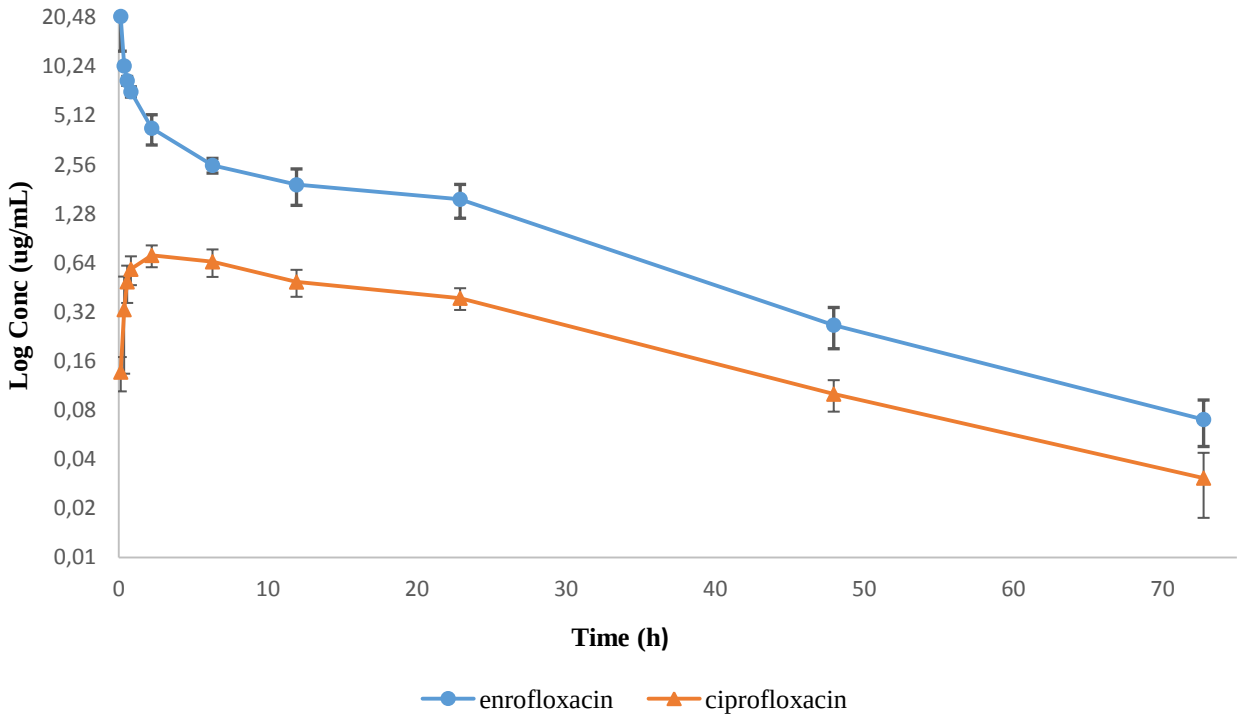

Fig. 2: average plasma concentration versus time profile of all 5 rhinoceros after successive IV and oral administration of enrofloxacin (circle) at 12.5 mg/kg and its ciprofloxacin (triangle) metabolite

4.5. Pharmacodynamics of enrofloxacin

The AUC_{24} (AUC_{enro24} + $AUC_{cipro24}$) after administration of 12.5 mg enrofloxacin/kg was 69.88 ± 14.94 µg/ml*h and 76.8 ± 8.86 µg/ml*h following intravenous and intravenous + oral enrofloxacin administration, respectively. Using the susceptibility breakpoint of 0.5 as the MIC value, the AUC_{24}: MIC ratio was 137.32 and 152.83, respectively. The C_{max}:MIC ratio in phase I and II was 28.54 and 41.52, respectively. The AUC_{24} and the AUC_{24}: MIC ratio after oral enrofloxacin administration could not be calculated. However, the semi-logarithmic plot (**Fehler! Verweisquelle konnte nicht gefunden werden.**) depicts a change in slope after 22.89 hours, which represents the pseudo C_{max} of 1.53 ± 0.37 µg/ml of the additive curve after subsequent intravenous and oral enrofloxacin administration. Thus, the estimated C_{max}: MIC ratio of the additive curve is 3.06.

5. Discussion

For this study we set out to determine the pharmacokinetics of enrofloxacin and ciprofloxacin in white rhinoceros. After intravenous administration, enrofloxacin was characterised by a half-life of 12.41 hours, which makes it the longest half-life following intravenous administration reported for this drug in any mammalian species thus far. In comparison, the half-life recorded in adult horses varies between 4.4 hours (Kaartinen, Panu, & Pyorala, 1997) and 6.15 hours (Peyrou, Bousquet-Melou, Laroute, Vrins, & Doucet, 2006). A more detailed evaluation of interspecies scaling of pharmacokinetic parameters, presented in the article '*Is the White Rhinoceros a Large Horse? The Use of Allometry and Pharmacokinetic Modelling to Evaluate the Importance of Interspecies Differences for One of Africa's Iconic Species'* (submitted for publication to PLOS ONE) demonstrated that the substantially longer half-life of enrofloxacin in the rhino cannot be solely explained by a lower metabolic rate relative to size (Sharma & McNeill, 2009). We suspect that the rhinoceros expresses a high degree of species-specific metabolic capacity that is neither readily extrapolated to their body size nor to their nearest related species being the horse. This difference would most likely result from distinctions in the cytochrome P450 (CYP450) enzyme content, in either enzyme type and/or relative concentrations (Leiberich, 2018).

Following intravenous administration of 12.5 mg enrofloxacin/kg with additional oral administration of 12.5 mg enrofloxacin/kg, the AUC extrapolated to infinity was 92.38 ± 12.14 µg/ml*h. The addition of oral enrofloxacin after an average of 10.06 hours resulted in a slight change in the profile compared to that of intravenous treatment alone. We estimated the fraction of absorption as the difference between the AUC_{tot} of the two phases. From this difference, we estimated the absolute bioavailability at 33.3 ± 38.34 %, which was highly variable between the treated animals. While the intrasubject variability is evident amongst other species (Haines, Brown, Gronwall, & Merritt, 2000; Nielsen & GyrdHansen, 1997), the oral absorption was substantially lower than that reported in domestic animal species and elephants (Bugyei, Black, & McEwen, 1999; Küng, Riond, & Wanner, 1993; Nielsen & GyrdHansen, 1997; Sanchez, Murray, Isaza, & Papich, 2005). In the horse, the bioavailability varied between 78.29 % and 55 % (Haines et al., 2000; Peyrou et al., 2006) in pigs between approximately 101 % in fasted and 83% in fed animals (Nielsen & GyrdHansen, 1997) while in dogs it varies between 63.22 % and 100 % (Bidgood & Papich, 2005; Küng et al., 1993).

The reason for the lower bioavailability is not known. However, since the study relied on the administration of the 10% oral solution of enrofloxacin manufactured for the administration in

the drinking water, non-specific binding to the molasses or feed or chelation to metal ions cannot be ruled out as the causative reason. Furthermore, based on conventional pharmacokinetic theory, low permeability of the gastrointestinal wall, metabolism of the drug in the gut wall, chemical degradation, physical inactivation, microbial transformation and hepatic first pass effect (Kwan, 1997; Peyrou et al., 2006) could have also contributed to a lowered oral bioavailability.

An important feature in the pharmacokinetics of enrofloxacin is the partial transformation into its active metabolite ciprofloxacin, which leads to a simultaneous circulation of both antimicrobials and an additive antimicrobial activity against certain bacteria such as *Pseudomonas aeruginosa* (Blondeau, Borsos, Blondeau, & Blondeau, 2012; Lautzenhiser, Fialkowski, Bjorling, & Rosin, 2001). In the rhino, plasma ciprofloxacin concentration reached 26.42 ± 0.05 % and 21.95 ± 0.02% of the plasma concentration of the parent drug. This compared favourably with the horse (20- 35%) (Kaartinen et al., 1997), sheep (26%) (Otero, Mestorino, & Errecalde, 2009) and goat (34%) (Rao et al., 2002). It was however higher than the 10% ciprofloxacin formation reported for the pig and the very low ciprofloxacin formation observed in the elephant (Nielsen & GyrdHansen, 1997; Sanchez et al., 2005). Despite the apparent similarity to the horse, an important difference can be seen with T_{max} of ciprofloxacin, which was in average 0.44 h ± 0.06 in the horse (Kaartinen et al., 1997) versus the substantially longer 2.1 ± 0.18 hours in the rhinoceros. This indicates once again that while the rhino has the requisite enzyme to metabolise enrofloxacin to ciprofloxacin, this enzyme system probably occurs at lower levels in the rhino. Further support for the limitation in metabolic capacity can be seen with the half-life of elimination of ciprofloxacin (11.62 ± 1.28 hours), which was considerably longer than the 5.1 ± 2.1 hours reported for the horse (Kaartinen et al., 1997).

Besides the assessment of the pharmacokinetic properties of enrofloxacin in rhinoceros, pharmacodynamic indices are valuable for the prediction of the ideal dose of the drug and are used to forecast antimicrobial success. Efficacy marker such as AUC_{24}: MIC and C_{max}: MIC have been identified for the assessment of the treatment outcome of the concentration dependent fluoroquinolones and their ratios have been found to be correlated with the success of an antimicrobial treatment (Hyatt, MCKinnon, Zimmer, & Schentag, 1995).

As a general MIC value for the evaluation of the efficacy marker in the rhinoceros, the published susceptibility breakpoint for enrofloxacin of 0.5 as determined by the CLSI (CLSI, 2015) was used. At this level, the AUC_{24}: MIC ratio was 137.32 and 152.83 after intravenous

and combined intravenous and oral enrofloxacin administration. These findings indicate that in both cases, enrofloxacin administration at a dose of 12.5 mg/kg exceeds the recommended ratio of 100 – 125 and leads to a bactericidal activity against susceptible bacteria. The C_{max}: MIC ratios after a single intravenous enrofloxacin injection and after the combined enrofloxacin treatment were 28.54 and 41.52, respectively. Those results largely exceed the recommended breakpoint values of 8 to 12 for a successful antimicrobial treatment. With both these surrogate markers being favourably, we conclude that intravenous enrofloxacin treatment would result in effective plasma concentrations. The oral curve did not add enough data for the calculation of the AUC_{24}: MIC ratio resulting from oral enrofloxacin administration only. However, the pseudo-C_{max} value of the additive curve estimated after subsequent intravenous and oral enrofloxacin administration was 1.53 ± 0.37 µg/ml, leading to a very low C_{max}: MIC ratio of 3.1. This ratio is much lower than the recommended ratio of 10 to 12 (Blaser, Stone, Groner, & Zinner, 1987) and indicates that the maintenance of the drug plasma concentration at a therapeutic level through additional administration of the 10 % oral solution of enrofloxacin, indicated for the use in chickens, turkeys and rabbits, at 12.5mg/kg is not feasible if one is aiming for a rapid, bactericidal effect with a low risk of emerging resistance.

Overall, due to the surprisingly low bioavailability in rhinoceros, the food-based medication with the 10% oral solution does not seem to be an option for a continued antimicrobial treatment. For the best and most reliable therapeutic outcome, a rhinoceros in a captive situation or one that can be kept in an enclosure for follow-up treatment could be re-sedated in form of a low dose butorphanol-based, standing sedation and enrofloxacin could then be re-injected intravenously, provided venous access is possible.

Conclusion

For this study, we assessed the pharmacokinetic properties and efficacy markers of enrofloxacin in white rhinoceros with the aim to evaluate the use of enrofloxacin for the treatment of poaching victims in particular, and any other white rhinoceros requiring antimicrobial treatment. The results were surprisingly different to those in domestic animal species with a half-life longer than previously recorded in combination with a considerably different oral bioavailability. While plasma concentrations after intravenous administration of 12.5 mg/kg injectable enrofloxacin resulted in surrogate markers above the recommended ratio of 125, the maintenance of the drug plasma concentration at a bactericidal level through the additional administration of the 10 % oral solution of enrofloxacin does not seem feasible.

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7. Competing interests

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

8. Author contribution statement

All authors have read and approved the final manuscript.

Author contributions:

ML: conceptualisation, formal analysis, funding, investigation, methodology, project administration, visualisation, writing – original draft preparation, validation

RK: formal analysis, validation

JM: conceptualization, funding, methodology, supervision

MH: conceptualization, methodology, supervision, investigation

VN: conceptualisation, funding, methodology, visualisation, validation, formal analysis, supervision

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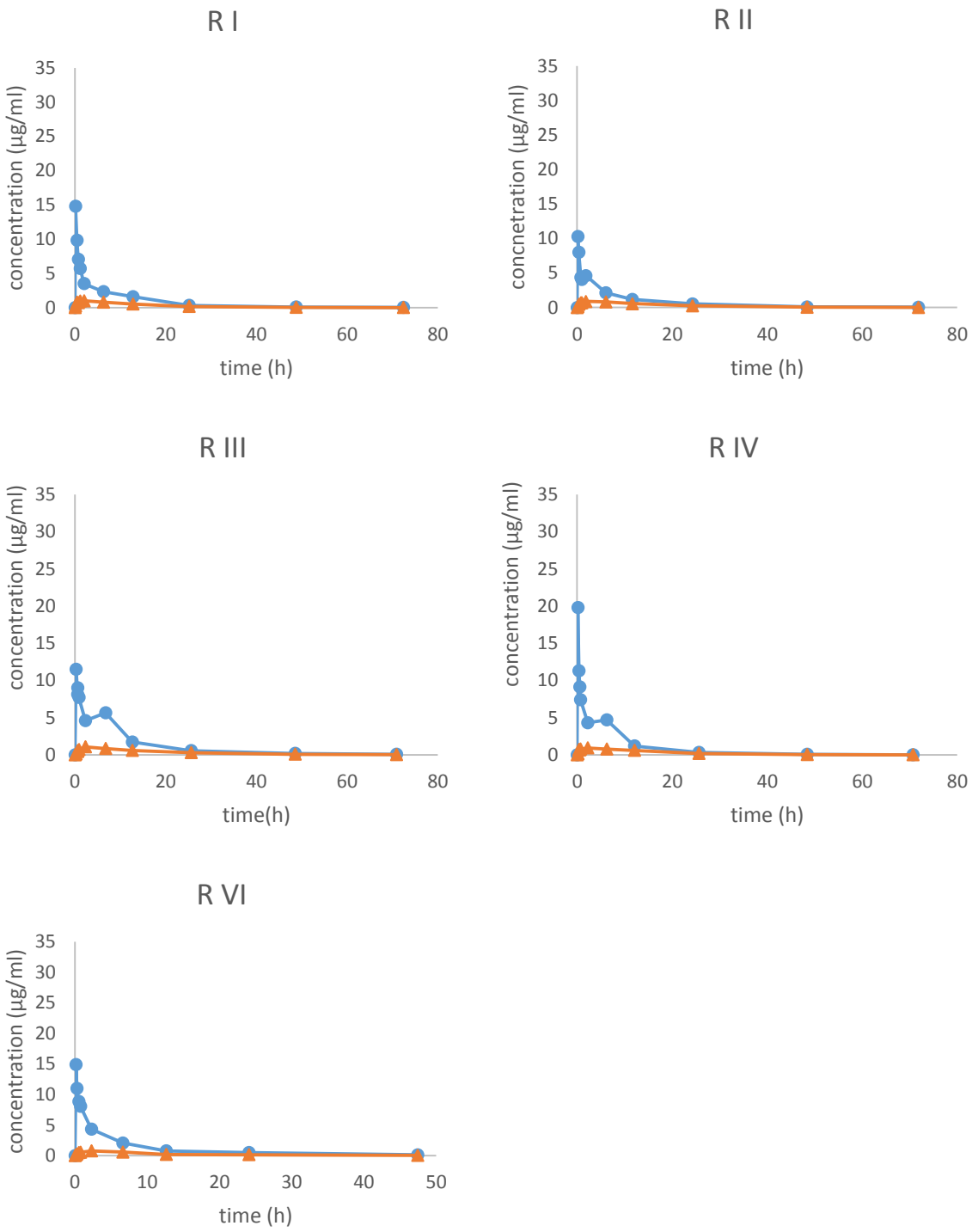
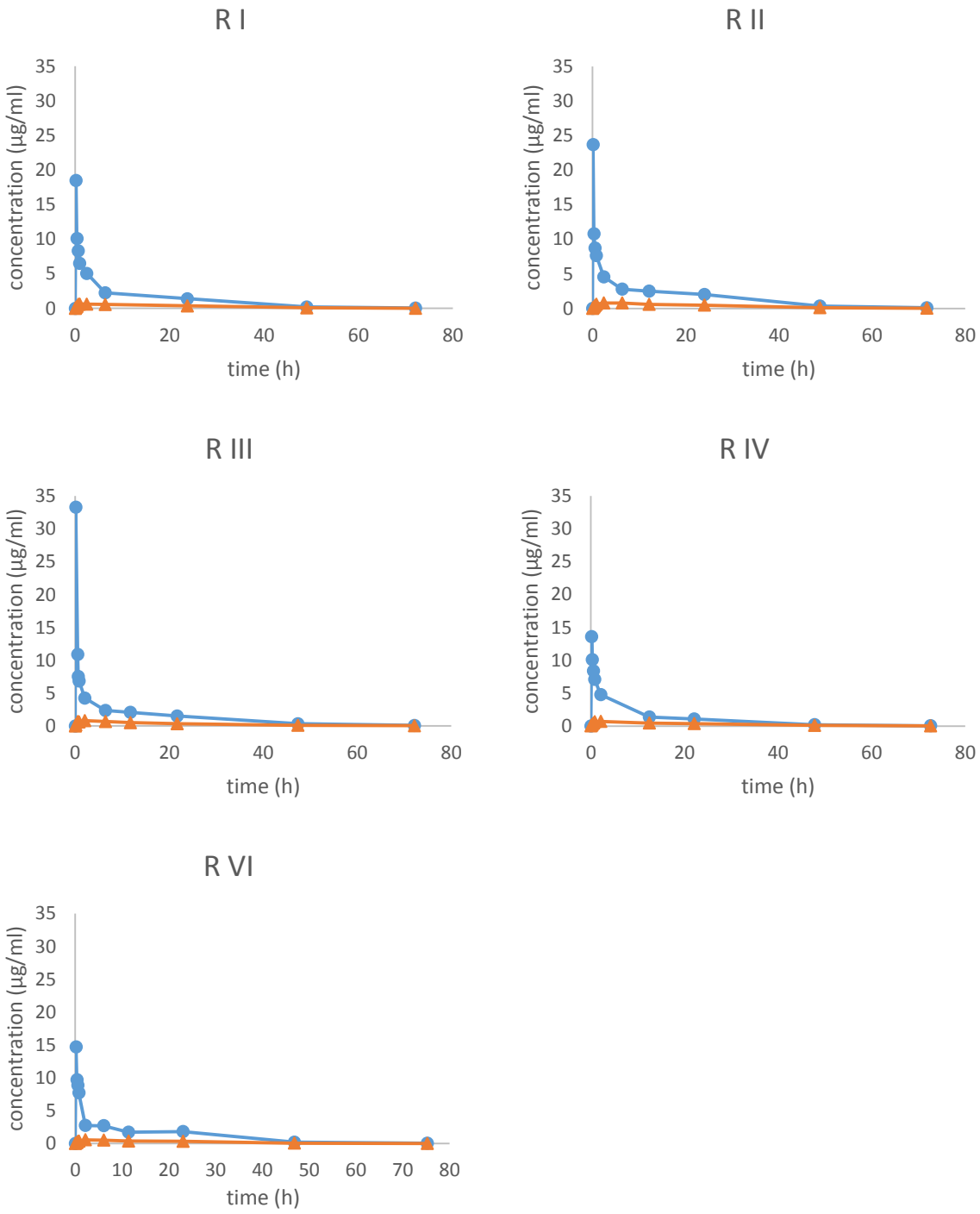
10. Supplementary information

S 1 table: Characteristics of the five white rhinoceros used in the pharmacokinetic study listed according to their age.

Age (months) First/second trial	Rhino	Sex	Weight (kg) First/ second trial	Dose of enrofloxacin administered (mg/kg)
13/15	Rhino I	Female	527/ 556	12.5
13/15	Rhino II	Male	477/ 538	12.5
17/19	Rhino IV	Male	505/ 551	12.5
18/20	Rhino III	Male	522/ 573	12.5
28/30	Rhino VI	Male	846/ 902	12.5



S 2 fig.: Plasma concentration versus time profile for each rhinoceros after IV administration of enrofloxacin (circle) at 12.5 mg/kg and its ciprofloxacin (triangle) metabolite



S 3 fig.: Plasma concentration versus time curve for each rhinoceros after IV and subsequent oral administration of 12.5 mg/kg enrofloxacin (circle) and its ciprofloxacin metabolite (triangle)