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Transdermal administration of flunixin meglumine results in systemic absorption in southern white rhinoceros (*Ceratotherium simum simum*)

Amanda Burke, DVM, DACVECC[1,2*]; Matthew E. Kinney, DVM, DACZM[3]; Kristen Phair, DVM, DACZM[3]; Matt Marinkovich, DVM, DACZM[1]; Larry J. Minter, DVM, MS, DACZM[4]; Danielle Mzyk, DVM, PhD, DACVCP[5]

[1]Department of Veterinary Services, San Diego Zoo, San Diego Zoo Wildlife Alliance, San Diego, CA
[2]College of Veterinary Medicine, University of California-Davis, Davis, CA
[3]Department of Veterinary Services, San Diego Zoo Safari Park, San Diego Zoo Wildlife Alliance, Escondido, CA
[4]Department of Animal Health, North Carolina Zoo, Asheboro, NC
[5]Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

*Corresponding author: Dr. Burke (aburke@sdzwa.org)

Objective
To assess the pharmacokinetics of a single application of transdermal flunixin meglumine in southern white rhinoceros (*Ceratotherium simum simum*).

Methods
Healthy adult white rhinoceros were prospectively enrolled in the study from June through September 2024. Baseline blood samples were collected prior to drug administration. A single (3.3 mg/kg) dose of commercially available transdermal flunixin meglumine was applied topically at 2 primary sites: the nuchal hump extending behind the ears and the skin overlying the spine from the shoulder to ischium. Blood samples were collected under behavioral restraint at 2 to 4 time points for each rhinoceros following a sparse-sampling model. Drug assay validation and plasma drug concentrations were determined using HPLC-MS-MS.

Results
Successful application of transdermal flunixin meglumine was achieved in 13 individuals. Twelve individuals were included in compartmental analysis. The time to maximum plasma concentration was 7.2 hours. The peak plasma concentration was 0.31 µg/mL. The elimination half-life was 28.6 hours. Mild behavioral changes were observed in 6 animals and included quiet mentation, rubbing at the application site, lethargy, and mild ataxia. All side effects were noted to be resolved 24 hours after drug application.

Conclusions
Transdermal flunixin meglumine was absorbed and measurable plasma concentrations achieved in all southern white rhinoceros. The side effects observed included short-duration behavioral changes.

Clinical Relevance
This study reports successful use and pharmacokinetic parameters of transdermal flunixin meglumine at 3.3 mg/kg when applied to the neck and topline in southern white rhinoceros.

Keywords: flunixin meglumine, southern white rhinoceros, transdermal, pharmacokinetics, *Ceratotherium simum simum*

Flunixin meglumine is an NSAID commonly used for analgesia.[1] It is currently the only FDA-approved NSAID for cattle in the US.[2,3] Flunixin meglumine provides its analgesic and anti-inflammatory effects through inhibition of the arachidonic acid cascade, thereby reducing the production of prostaglandins and thromboxane, which are key mediators of inflammation.[1] In domestic species, flunixin meglumine has been used for treatment of pain and inflammation associated with lameness, mastitis, bovine respiratory disease, and endotoxemia.[4–7] Additionally, it has been employed in nondomestic hoofstock species for treatment of osteoarthritis, colic, and trauma.[8,9]

The southern white rhinoceros (*Ceratotherium simum simum*) is classified as near threatened by the

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International Union for the Conservation of Nature.[10] Major threats to wild populations include increased poaching pressure and illegal wildlife trade, which have worsened since the COVID-19 pandemic.[10] Managed populations play a critical role in conservation, where collaboration with veterinary care is essential to maintain health and welfare.[10] Commonly encountered health concerns in managed white rhinoceros populations include soft tissue injuries, horn avulsions, and various infectious diseases, for which analgesic therapy is commonly employed.[11] An online survey performed in 2016 of the American Association of Zoo Veterinarians Listserv indicated NSAIDs (eg, flunixin meglumine, phenylbutazone, firocoxib, meloxicam) to be the most commonly used analgesic group in rhinoceroses.[12] Pharmacokinetic investigation of orally administered flunixin meglumine in the white rhinoceros has demonstrated sufficient absorption and metabolism of a single oral dose, resulting in plasma concentrations potentially sufficient to produce analgesia, when compared to domestic equine pharmacokinetics-pharmacodynamics.[8,13] Unfortunately, the most commonly reported complication of oral administration is taste aversion, which can result in low administration success.[12] Taste aversion to the oral formulation, frequent disease-related anorexia, and volume limitations of IM administration emphasize the need to investigate alternative drug administration routes for flunixin meglumine in this species.

A formulation of flunixin meglumine designed for transdermal use has been available since 2018. Application in juvenile cattle using a single transdermal dose of 3.3 mg/kg achieved rapid absorption with a longer half-life compared to IV administration.[2,4] In horses, transdermal flunixin meglumine use at a dose of 500 mg/horse produced plasma drug concentrations that achieved anti-inflammatory effects for 24 to 72 hours.[14] The absorption half-life was not calculated in that study, but the average concentration 15 minutes after application was 1.17 ± 67.7 ng/mL. The mean peak concentration (C_{max}) reported in horses was 0.5 µg/mL, occurring between 8 to 12 hours (mean, 8.67 hours).[14] Unique features of rhinoceros skin that may affect absorption include a dense capillary network and collagenous dermal "armor," which can be up to 4.5-cm thick.[15] These anatomic variations may limit transdermal uptake. A literature search using Google Scholar, Commonwealth Agricultural Bureaux International, and PubMed and the keywords rhinoceros, pharmacokinetics, and transdermal flunixin meglumine did not obtain any results. Similarly, searches for other thick-skinned taxa, including elephants and hippopotamus, did not yield any results. The objective of this study was to investigate the pharmacokinetics of a single dose of transdermal flunixin meglumine in southern white rhinoceros using published dosages in cattle with strategic selection of the drug application site.[2,16] We hypothesized that drug application primarily over the neck, and secondarily over the topline, would result in measurable plasma drug levels.

Methods

This study was approved by the IACUCs from both study locations, the San Diego Zoo Safari Park (No. 23–022) and North Carolina Zoo (No. 2024–2). At both institutions, rhinoceros were housed outside in mixed-species habitats, with access to covered spaces and exposed to ambient temperatures. Application and sample collection occurred for all individuals from June 20, 2024, through September 19, 2024. During this time period, average ambient temperatures ranged from 17 to 28 °C and 18 to 31 °C in California and North Carolina, respectively. Healthy adult rhinoceros were eligible for inclusion. Rhinoceros were excluded if they were pregnant or had received any NSAID within the 4 weeks prior to the start of the study. Body weights were obtained within a 7-day period before drug application for accurate dosage calculation.

Prior to drug application, all study animals had their skin dry brushed in 2 major sites to remove dirt and debris that might impede drug absorption. The primary application site was prepared first and included the skin over the dorsolateral aspects of the neck, behind the ears, and over the cervical hump (Figure 1). The secondary site was also dry brushed and encompassed the skin over the thoracolumbar spine and epaxial muscles (Figure 2). Water and other cleaning solutions were not utilized so as to avoid interference with drug absorption. The skin was kept dry for 6 hours prior to drug application. Skin cleanliness after dry brushing was assigned a score from 0 to 3 (0 = < 25% of the total surface area of the primary and secondary sites cleaned, 1 = 25% to 50% of the total surface area of the primary and secondary sites cleaned, 2 = 50% to 75% of the total surface area of the primary and secondary sites cleaned, and 3 = 100% of the total surface area of the primary and secondary sites cleaned). Any individuals receiving a score of 0 did not undergo drug application. Wildlife care specialists monitored the treated individuals for the duration of the study and were not blinded to study inclusion.

Topical administration of 3.3 mg/kg of commercially available flunixin meglumine (Banamine Transdermal Pour-On) was applied over the primary site (Figure 1). Extralabel drug use was performed with the provisions of AMDUCA and Title 21, Part 530 of the Code of Federal Regulations. In some cases, due to large drug volume, application was also performed over the secondary site as previously described.

Venipuncture was performed via the radial, pedal, or auricular veins using 18- to 22-gauge needles, extension lines, and 3- to 12-mL syringes. Baseline plasma samples were obtained prior to drug application. After transdermal flunixin meglumine application, plasma collection was performed at scheduled time points for each animal based on a sparse-sampling model. A sparse-sampling model was utilized due to the limited number of samples that could practically be obtained from each individual. Each individual was randomly assigned 2 to 4 time points at 1, 2, 4, 6, 8, 12, 24, or 48 hours. The target blood collection volume was 2 mL/time point. Blood samples

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were transferred into lithium heparin tubes and kept on ice until processing. Processing was performed within 2 hours and consisted of centrifugation to isolate plasma into cryovials, which were stored at −20 to −80 °C until pharmacokinetic analysis.

Plasma sample preparation

One hundred microliters of rhinoceros plasma was pipetted into the 2.0-mL microcentrifuge tubes (Thermo Scientific), and 500 µL of 4% phosphoric acid prepared in water was added to each tube to pretreat the plasma. An Oasis Prime HLB 96-well µElution Plate (Water Corp) was used for solid-phase extraction. The plate was prepared by conditioning the plate with 500 µL of MeOH followed by 500 µL of ultrapure water. Next, 600 µL of pretreated plasma was loaded on the Prime HLB µElution Plate and passed through the plate by using a positive pressure manifold (Otto SPEcialist; Waters Corp). After that, the plate was washed with 600 µL of 5% MeOH in water. The analyte was eluted into a clean 96-well sample plate (700-µL round, 96-well samples plate; Waters Corp) with the addition of 100 µL of an elution solution (70:30 acetonitrile to MeOH). The eluents (0.5 µL) were injected into the instrument for analysis. Standards from 0.0005 to 1 µg/mL were prepared using blank rhinoceros plasma. The calibration curve of flunixin meglumine was fitted with a weighted (1/concentration) linear equation (Targetlynx software, version 4.0; Waters Corp). The calibration range of 0.0005 to 1 µg/mL was linear, with a coefficient of determination, R^2, greater than 0.99. Each calibration standard concentration could be back calculated to within 15% of the true concentration. To validate the analytical method, a total of 5 replicates at concentrations of flunixin meglumine (0.001, 0.003, 0.03, and 0.7 µg/mL) were performed on the same day using the same batch to assess intraday precision. The intraday precision and accuracy were calculated. The precision was 3.24% to 17.81%, and recovery was 93.0% to 109.0%. The limit of detection and limit of quantification were recognized as 0.0005 µg/mL and 0.001 µg/mL, respectively, based on interday precision and accuracy, signal-to-noise ratio, and chromatograph.

UPLC-MS-MS conditions

Chromatographic separation was performed by a gradient elution on the ACQUITY UPLC BEH Phenyl 1.7-µm Column (2.1 X 100 mm) with a VanGuard precolumn (Waters Corp). The mobile phase solvents consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), and the flow rate was 0.4 mL/min for 5 minutes. The gradient program mobile phase conditions were 70% of A and 30% of B for the first 2.5 minutes, then changed linearly to 10% of A and 90% of B from 2.5 to 3.5 minutes, then immediately back to 70% of A and 30% of B from 3.5 to 5 minutes to re-equilibrate at the initial conditions. The column temperature and the autosampler temperature were maintained at 35 and 25 °C, respectively. The positive electrospray ionization was used with the multiple reactions monitoring. The tune page source voltages were 3.5 kV and 50 V for the capillary and cone, respectively. The source desolvation temperature was 350 °C. The source desolvation gas flow and cone gas flow were 650 and 50 L/h, respectively. The mass spectrometry file cone voltage setting was 44 V with a collision energy setting of 34 and 22 V. Argon was used as the collision gas, and nitrogen was used as the desolvation and cone gases. Quantification was performed using the transition parent (m/z) 297.10 and daughter (m/z) 263.99 and 279.09, with a retention time of 2.43 minutes.

Pharmacokinetic analysis

Plasma drug concentrations versus time were plotted on linear and semilogarithmic graphs for visual inspection and assessment of the best model for pharmacokinetic analysis. Analysis of the curves and pharmacokinetic modeling were then performed using a commercial pharmacokinetic program (Phoenix WinNonlin, version 8.0; Certara Inc). Compartmental analysis of the data was performed using a weighting factor of $1/Y^2$, where Y is the predicted flunixin meglumine plasma concentration. The primary parameters were calculated using the following formula:

$$C = \frac{k_{01}FD}{V(k_{01} - k_{10})}[e^{-k_{10}t} - e^{-k_{01}t}]$$

where C is the plasma flunixin meglumine concentration, t is time after administration, k_{01} is the non-IV absorption rate assuming first-order absorption, k_{10} is the elimination rate constant, V is the apparent volume of distribution, F is the fraction of drug absorbed, and D is the non-IV dose. Secondary parameters calculated from the model included the C_{max}, time to C_{max} (t_{max}), area under the plasma concentration-versus-time profile, PO, apparent clearance, and respective absorption and elimination half-lives. Absolute bioavailability was not determined due to the lack of IV administration data.

Statistical analysis

A naïve averaged analysis was used to obtain initial estimates, which were used as input for the population pharmacokinetic analysis. This analysis produces the typical value for the population parameters (fixed effects) as well as estimates for the between-subject variability (random effects). From these initial estimates, the nonlinear mixed-effects model was fitted to these data (Phoenix NLME, version 8.3; Certara Inc). A nonlinear mixed-effects model was selected given the sparse and unbalanced pharmacokinetic data, allowing for evaluation of both population-level parameters and interindividual variability.

Various models were tested with different error structures to determine the best fit base model. Final model selection was based on goodness-of-fit plots, diagnostic plots of residuals, scatter plots of predicted-versus-observed values, and statistical significance ($P < .05$) between models using the minimum value of the objective function.

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Interindividual (between-subject) variability (variance of a parameter among different subjects) was expressed using an exponential error model according to the following equation:

$$Pi = \theta Px \exp(\eta iP)$$

where P is the pharmacokinetic parameter of interest for the individual (i); θP is θ, or the typical value (fixed effect) for the population estimate of the parameter of interest, *x*exp is the exponential function; and ηiP is the η (random effect) for the interindividual (between-subject) differences of the parameter of interest. The η values were assumed to be independent and have a normal distribution with a mean of zero and variance of ω^2. A multiplicative model described the residual random variability (ε) of the data, where ε is the residual intrasubject (within subject) variability with a mean of zero and a variance of σ^2, according to the following equation:

$$C_{obs} = C_{predx}(1 + \varepsilon)$$

where C_{obs} is the observed concentration for the individual, and C_{predx} is the model-predicted concentration plus the error value (ε).

Results

Thirteen healthy adult southern white rhinoceros were enrolled in this study, 4 individuals from the North Carolina Zoo and 9 individuals from the San Diego Zoo Safari Park. Health was assessed using physical examination, CBCs, and biochemistry profiles prior to enrollment in the study. The individuals included in the study comprised 1 male and 12 females. Ages ranged from 4 to 30 years, with a mean age of 16.3 years. Body weights ranged from 1,586 to 2,323 kg, with a mean weight of 1,930 kg **(Figure 1)**.

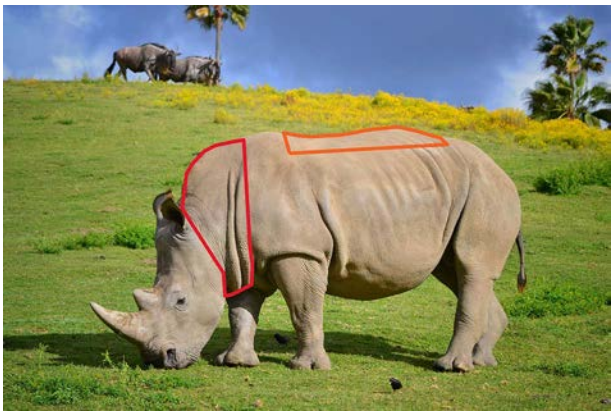

Figure 1—Demonstration of the primary and secondary transdermal drug application site in a southern white rhinoceros (*Ceratotherium simum simum*). The area outlined in red, encompassing the area over the dorsolateral aspects of the neck, behind the ears, and over the cervical hump, was the primary and preferred application site. The area outlined in orange, encompassing the area over the thoracolumbar spine (colloquially referred to as the "topline"), was the secondary application site.

Transdermal flunixin meglumine was successfully applied in all study individuals. Skin-cleansing scores were noted in 12 of 13 individuals, with 10 assigned a score of "3" and 2 assigned a score of "2." The individual without a documented cleansing score was included in the pharmacokinetic analysis. Successful application of topical flunixin meglumine was achieved in all individuals. Nine individuals utilized only the primary (preferred) site of application, whereas 4 individuals required use of both the primary and secondary application sites. The actual dosage utilized ranged from 3.26 to 3.31 mg/kg (mean, 3.29 mg/kg).

A total of 13 baseline plasma samples were obtained, and 40 postapplication plasma samples were acquired, ranging from 1 to 48 hours **(Figure 2; Table 1; Supplementary Table S1)**. One rhino was removed from compartmental analysis for initial estimates because only 2 plasma samples (1 and 3 hours) were collected, and this individual was the only animal sampled at 3 hours. This animal's plasma concentrations were used in the subsequent nonlinear mixed-effect analysis.

Pharmacokinetic parameters are listed in **Table 2**. The t_{max} was 7.22 hours. The C_{max} was 0.31 µg/mL. The area under the curve for the plasma concentration-versus-time profile of a single dose was 15.2 µg·h/mL. The absorption half-life was 1.65 hours, and the elimination half-life was 28.6 hours. The apparent clearance from plasma was 0.21 L/h/kg.

Changes in behavior were observed in 6 of the 13 individuals. One individual was noted to have a quieter mentation 2 hours after transdermal application, which was noted to normalize 24 hours after application. Excessive rubbing of the neck was observed in

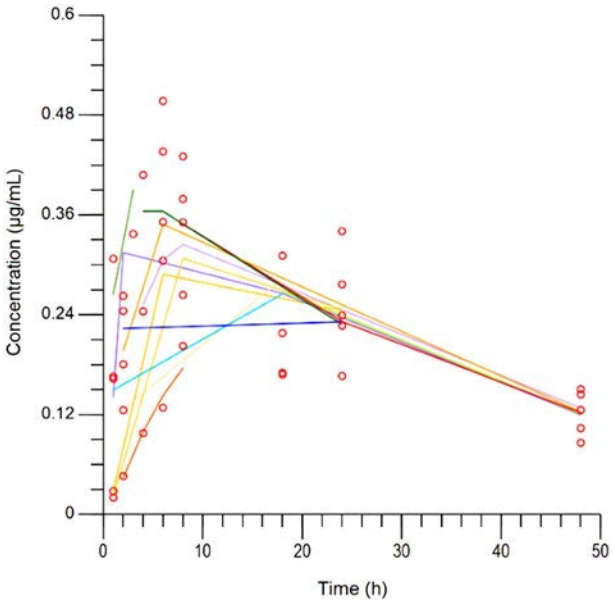

Figure 2—Plot of plasma flunixin meglumine concentrations for individual southern white rhinoceros (n = 13) over time. Each line represents an individual rhinoceros. Each open circle represents an actual data point. The fitted line shown for each rhinoceros is overlaid on the actual data points.

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Table 1—Mean plasma concentrations of flunixin meglumine per time point for southern white rhinoceros (n = 13) treated with a single dose (3.3 mg/kg) of transdermal flunixin meglumine.

Time (h)	No. of rhinoceros sampled	Mean (µg/mL)	SD (µg/mL)
1	5	0.14	0.12
2	5	0.17	0.09
3	1	0.34	—
4	3	0.25	0.16
6	4	0.34	0.14
8	6	0.33	0.09
18	4	0.22	0.07
24	5	0.25	0.06
48	5	0.12	0.03

Rhinoceros were sampled from June through September 2024 based on a sparse-sampling model.

Table 2—Single-dose pharmacokinetic parameters derived from the mixed-effects model for flunixin meglumine in southern white rhinoceros administered a single transdermal dose of 3.3 mg/kg.

Parameter	Units	Estimate	Standard Error	CV%
K_a	1/hr	0.56	0.12	21.7
V/F	L/kg	8.0	0.49	6.2
Cl/F	L/hr/kg	0.21	0.02	7.4
T_{max}	hr	7.22	—	14.9[a]
C_{max}	µg/ml	0.31	—	8.8[a]
AUC	µg*hr/mL	15.2	—	14.9[a]
$K_{01}t$	hr	1.65	—	26.6[a]
$K_{10}t$	hr	28.6	—	24.2[a]

AUC = Area under the concentration-versus-time curve. CL/F = Apparent clearance per fraction absorbed. C_{max} = Maximum plasma concentration. $K_{01}t$ = Absorption half life. $K_{10}t$ = Elimination half life. K_a = Absorption rate constant. T_{max} = Time to maximum concentration. V/F = Volume of distribution per fraction absorbed.

[a]CV% generated from compartmental model.

1 rhino 16 hours after application. One individual was noted to appear hyperreactive to noise stimuli 18 hours after application. Lethargy was noted in 3 individuals between 18 and 24 hours after application. Additionally, 1 of these individuals was noted to have mild ataxia 16 to 19 hours after application. Lethargy and ataxia resolved 24 hours after application.

Discussion

This study reports pharmacokinetic data of a single dose of transdermal flunixin meglumine in a zoo-managed population of healthy southern white rhinoceros. Transdermal flunixin meglumine has been evaluated in domestic species, including goats, cattle, swine, donkeys, and alpacas.[5,17–20] Identical dosages (3.3 mg/kg once or every 24 hours) produced different pharmacokinetic parameters compared to those observed in this study.[5,17–20] In southern white rhinoceros, the t_{max} was 7.2 hours, which is longer than reported in adult cattle and calves (2.8 and 2.14 hours, respectively) but shorter than goats and alpacas (11.4 and 13.5 hours, respectively). The C_{max} in this study was 0.31 µg/mL (310 ng/mL), which is higher than reports in sows, goats, and alpacas but lower when compared to adult cattle and calves.[5,17–20]

Transdermal drug efficacy depends on skin permeability, which can vary based on location on the body.[21,22] Additionally, the presence of hair or fiber can impact transdermal drug absorption as well as dermal fat concentrations.[18,20] Moisture and water on the skin have also been shown to impact absorption, and it is therefore recommended to keep skin clean and dry for 6 hours after application.[23] In rhinoceros, the thickest layer of skin is the stratum corneum, the outermost layer of the epidermis.[15] First-generation transdermal medications diffuse through the stratum corneum and hair follicles via lipophilic vehicles to reach the vascular dermis and ultimately achieve systemic absorption.[22] Despite the increased epidermal thickness, rhinoceros possess a dense network of arteries and arterioles in the superficial dermis, including in the nuchal region.[15] These features likely explain the successful absorption observed in this study as well as the differences observed in the t_{max} and C_{max} in other domestic animal pharmacokinetic studies.

A pharmacokinetic study[8] in southern white rhinoceros evaluated oral flunixin meglumine administered at 1 mg/kg. In that study,[8] the t_{max} was 3 hours, shorter than this study (7.2 hours), and the C_{max} was higher at 1,207 ng/mL compared to this study (310 ng/mL). Additionally, the half-life of oral flunixin meglumine in that study was 8.3 hours in comparison to 28.6 hours in this study. The pharmacokinetics of oral flunixin meglumine has also been evaluated in Asian and African elephants, where a single 1.5-mg/kg oral dose produced maximum plasma concentrations of 7,200 ng/mL in Asian elephants and 4,400 ng/mL in African elephants, higher than both this study and the previously mentioned oral flunixin meglumine study[8] in southern white rhinoceros.[9] Differences in oral absorption between southern white rhinoceros and elephants is likely a reflection of gastrointestinal tract differences (eg, transit time, tract length, pH variations).[24] Pharmacokinetics comparison between oral and transdermal absorption of flunixin meglumine in white rhinoceros reflect a faster t_{max}, shorter half-life, and higher C_{max} when administered orally.[8] This is likely due to improved bioavailability via the oral route of administration compared to transdermal administration in this species; however, future studies are needed to confirm this assumption.

There are no published reports for therapeutic plasma concentrations of flunixin meglumine in rhinoceros. This study did not evaluate pharmacodynamics or measurements of inflammatory biomarkers. However, reports of transdermal flunixin meglumine and evaluation of pharmacodynamics and biomarker measurement have been performed in goats, alpacas, and horses.[14,17,18] In goats, a partial reduction in prostaglandin E2 was observed, with a mean 80% inhibitory concentration of 280 ng/mL.[17] Similar observations in alpacas were observed, with

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a mean 80% inhibitory concentration at 230 ng/mL.[18] In experimentally induced arthritis in horses, therapeutic plasma concentrations ranged from 200 to 900 ng/dL.[13] Based on these reports, plasma concentrations observed in this study may reflect appropriate levels for anti-inflammatory effects for up to 30 hours. Conversely, pharmacodynamics of transdermal flunixin meglumine failed to show changes in inflammatory biomarkers in sows and no changes in vital parameters or prostaglandin E2 after castration in goats.[7,16] Future studies evaluating pharmacodynamics and bioavailability are necessary to determine therapeutic efficacy in white rhinoceros.

Only clinically apparent adverse effects were noted in this study. Adverse effects were limited to behavioral changes, including lethargy, rubbing at the application site, and ataxia, all of which resolved 24 hours after initial drug application. Previous studies[14,16–19,25] of transdermal flunixin meglumine at various dosages, including the dosage used in this study, have not been associated with adverse effects. Similarly, studies[8,9] evaluating oral flunixin meglumine in elephants (*Loxodonta africana* and *Elephas maximus*) at 0.8, 1.5, and 1.1 mg/kg and white rhinoceros at 1 mg/kg did not result in any adverse effects. A study[26] in American bullfrogs evaluating transdermal flunixin meglumine observed mild cutaneous lesions, but no other adverse effects were observed. Causes for the adverse effects observed in this study were not determined. All side effects occurred at a single institution, and therefore observer bias is possible. The product used in this study is a propylene glycol–based formulation containing pyrrolidone, menthol, red dye, and glycerol monocaprylate.[25] Propylene glycol is widely accepted to have a low level of toxicity, is found in numerous medications, and is poorly absorbed through skin; however, reported side effects when administered at toxic doses include CNS depression.[27] The rhino that exhibited ataxia received 0.03 g/kg of propylene glycol, well below any dosage reported to cause side effects in animals and people (1.2 to 15 g/kg).[27] Other adverse effects reported with NSAID administration include gastrointestinal injury (eg, ulceration, alerted microbiota), idiosyncratic hepatic injury, renal insufficiency secondary to modulation of renal blood flow, and prolonged bleeding times due to prevention of platelet aggregation.[28,29] These side effects were not apparent during this study; however, further investigation is warranted. Next steps should include multidose trials, gastric mucosal investigation using traditional or capsular endoscopy, measurement of renal biomarkers, platelet function testing, buccal mucosal bleeding time evaluation, fecal occult blood testing, and fecal microbiome tracking. Additionally, a double-blinded study would eliminate potential observer bias.

There are limitations to this study. First, this study evaluated a single route of drug application and a single dose. Human studies[30] evaluating peripheral and central sampling locations have shown differences in C_{max} and t_{max}. A pilot study[31] in 1 pig compared flunixin concentrations from the femoral artery, jugular vein, and auricular artery following intranasal administration and found that while the t_{max} was similar for all sites, there were likely differences in the concentration values. This provides some evidence that proximity to dosing site may contribute to the variability in reported plasma flunixin concentrations. Future studies could compare the administration of IV and topical application as well as serial topical applications in different age classes and sexes in this species. Second, evaluation of inflammatory markers, such as fibrinogen, protein C, or prostaglandins (eg, prostaglandin E2), to better characterize flunixin meglumine pharmacodynamics in white rhinoceros should be considered in future studies. Third, the small sample size and the use of a sparse-sampling strategy was a necessary choice driven by both animal welfare and practical field constraints inherent to pharmacokinetic studies in rhinoceros. The sampling time schedule was designed to ensure that samples from 3 to 4 animals were collected per time point between zero and 48 hours after drug administration. These methods have been successfully used for studies[32,33] involving sparse sampling of zoo and exotic species. This approach allows for sparse sampling for each subject and mixed-effects modeling to estimate the fixed effects (depicting pharmacokinetic parameters) and random effects (to account for interindividual variation). Since the limited number of samples per individual was a logistical necessity, a nonlinear mixed-effects population model was chosen to make the most efficient and scientifically valid use of these data. Fourth, in rhinoceros, hepatic biotransformation results in secondary metabolites, including 5-OH flunixin, which has been previously quantified at detectable concentrations following oral administration of 1 mg/kg in white rhinoceros.[8] Additional studies should be performed to quantify 5-OH and any additional metabolites as well as evaluate if these metabolites are pharmacologically active. Another limitation is the high interindividual variation due to the use of multiple study participants. To limit this influence, plasma samples could be obtained from the same individual. Finally, this is the first report of the successful absorption of transdermal flunixin meglumine in thick-skinned mammals, and similar pharmacokinetics should be investigated in mammals with similar cutaneous anatomy.

In conclusion, our study reports successful absorption and measurable plasma drug concentrations after a single transdermal dose of flunixin meglumine in southern white rhinoceros. Side effects were observed, including quiet mentation, rubbing of the application site, lethargy, and ataxia. All side effects were resolved within 24 hours of drug application and were considered mild. Based on pharmacodynamics evaluation in other species, the dosage used in this study may reflect therapeutic concentrations; however, future studies are needed to establish this. In light of these findings, the use of a single dose of transdermal flunixin meglumine at 3.3 mg/kg in southern white rhinoceros over the primary application site may have clinical utility in providing analgesia to hyporexic rhinoceros.

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ORCID

Danielle Mzyk https://orcid.org/0000-0002-9834-2089

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Supplementary Materials

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Supplementary Table S1. Individual plasma concentrations (μg/mL) for southern white rhinoceros (n=13) treated with a single dose of transdermal flunixin meglumine (3.3 mg/kg). Please note that rhinoceros identification numbers 1-4 received drug application in both the primary and secondary application sites due to large drug volume. Rhinoceros identification numbers 5-13 received drug application to the primary application site only.

	Rhinoceros Identification												
Time (hour)	1	2	3	4	5	6	7	8	9	10	11	12	13
1	0.17				0.16				0.31	0.02	0.03		
2	0.26					0.24	0.05	0.13				0.18	
3									0.34				
4		0.24	0.41				0.10						
6		0.35	0.44				0.13					0.50	
8		0.26	0.43				0.20			0.35	0.31		0.38
18	0.31			0.17	0.16			0.17					
24			0.17			0.34				0.28	0.23		0.24
48	0.15	0.10		0.09								0.14	0.13