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Pharmacokinetics of a single dose of flunixin transdermal formulation in American bullfrogs (*Lithobates catesbeianus*)

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Objective
To determine the pharmacokinetics of a single dose of flunixin transdermal formulation in American bullfrogs (*Lithobates catesbeianus*).

Methods
Clinically healthy, purpose-bred adult bullfrogs housed at the North Carolina State University College of Veterinary Medicine were enrolled in a sparse-sampling population study. Frogs were administered 3.3 mg/kg transdermal flunixin meglumine (Banamine Transdermal; Merck Animal Health) on the dorsum via micropipette under manual restraint in July of 2022. Frogs were maintained in individual containers out of water for 4 hours and randomly assigned to 2 of the following venipuncture time points: 1, 2, 4, 8, 12, or 24 hours, with 7 frogs sampled per time point. Blood was collected from the popliteal sinus. Ultra performance liquid chromatography–tandem mass spectrometry was used to determine plasma flunixin concentrations. Data were analyzed using noncompartmental analysis.

Results
Flunixin was detected in all samples collected from 21 bullfrogs (9 males and 12 females). A mean peak plasma concentration of 2.39 µg/mL was reached between 1 and 2 hours. The elimination half-life was 15.0 hours. Plasma concentrations were similar across individuals at 1, 2, and 4 hours (range at 1 and 2 hours, 2.32 to 2.55 µg/mL) but were variable at 8, 12, and 24 hours (range at 24 hours, 0.16 to 1.79 µg/mL). Mucus and/or epithelial loss was noted at the drug application site in 18 of 21 frogs. No additional clinical signs or mortality occurred.

Conclusions
Transdermal flunixin was systemically absorbed, and plasma concentrations exceeded established therapeutic ranges in other species. Most frogs developed mild cutaneous lesions.

Clinical Relevance
Transdermal flunixin was detected in plasma for 24 hours; however, variability in plasma concentrations over time and topical side effects may limit its use.

Keywords: amphibian, analgesia, flunixin meglumine, NSAID, transdermal

Amphibians are a diverse group with more than 8,000 extant species, the majority of which are anurans, or frogs and toads. Anurans are commonly maintained in zoos, aquaria, research institutions, and households and routinely require analgesia for painful conditions or procedures. In mammals, NSAIDs are a mainstay of analgesic therapy. Nonsteroidal anti-inflammatory drugs decrease prostaglandin synthesis via competitive inhibition of cyclooxygenase enzymes and, as a result, provide anti-inflammatory, antipyretic, and analgesic effects.[1] It is presumed that this mechanism is conserved in anurans, and a prior study in American bullfrogs (*Lithobates catesbeianus*) demonstrated decreased serum prostaglandin levels in response to meloxicam administration postinjury.[2] Nonsteroidal anti-inflammatory drugs are widely used in clinical settings across taxa, including anurans, and offer

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multiple benefits to patients and prescribers as they are accessible, generally inexpensive, not federally controlled, and can be delivered via multiple routes of administration (eg, PO, SC, IM, and IV). Despite their use, pharmacokinetic and pharmacodynamic data for NSAIDs in anurans are limited.

The unique semipermeable skin of amphibians is a major interface for gas, water, and electrolyte exchange and can be leveraged for topical drug administration.[3–7] This technique avoids the pain and stress associated with injections and may be particularly advantageous in small, delicate species in which safe handling and repeated injections are challenging. Preliminary pharmacokinetic studies have begun to explore the topical application of NSAIDs in anurans, and results have been mixed. In smoky jungle frogs (*Leptodactylus pentadactylus*), ketoprofen at 1 mg/kg administered topically was detectable in plasma for 24 hours, but topical meloxicam at 0.2 mg/kg was not detectable at any time point (3, 8, or 24 hours).[8] That study used commercial NSAID formulations intended for injection and doses extrapolated from other taxa, highlighting that these 2 factors (ie, formulation and dosing) might impede the success of transdermal NSAID administration in amphibians.[8–10]

Conveniently, the NSAID flunixin meglumine (Banamine Transdermal; Merck Animal Health) has recently been formulated and made commercially available in the US specifically for transdermal use in cattle. This product is FDA approved for fever associated with bovine respiratory disease or mastitis and control of pain from foot rot in cattle. A single study investigating the pharmacokinetics of this product in a toad species demonstrated favorable results.[11] When administered topically to the dorsum of marine toads (*Rhinella marina*), transdermal flunixin meglumine (3.3 mg/kg) was rapidly absorbed, and peak plasma concentrations (C_{max}, 6.31 µg/mL) exceeded an established therapeutic concentration for cattle (C_{max}, 1.08 µg/mL).[11–13] While the concurrent pharmacodynamics are not known, this study builds support for the use of transdermal flunixin meglumine in other anuran species.

Due to the paucity of species-specific pharmacologic data, veterinarians currently rely on extrapolation from other taxa and anecdotal knowledge when choosing amphibian analgesic protocols. However, indiscriminate application of the aforementioned marine toad data to all anuran species, particularly true frogs, is not ideal and could result in inadvertent morbidity or mortality.[4] True frogs are semiaquatic and possess moist, smooth skin covered in a lipid-containing mucus layer.[14–17] In contrast, toads (including the inaptly named marine toad) are primarily terrestrial and have dry, bumpy skin without a mucus layer.[17] Thus, uptake and pharmacokinetics of transdermal flunixin may be vastly different between toads and true frogs, and species-specific investigation is warranted. The objective of this study was to assess the pharmacokinetics of a single dose of topical transdermal flunixin meglumine in American bullfrogs (*Lithobates catesbeianus*).

Methods

Animals

This study was approved by the North Carolina State University IACUC (19-614-O). Twenty-one mature, clinically healthy bullfrogs (9 males and 12 females, all approx 2 to 3 years of age) were obtained from a commercial breeder (Frog Pharm). Their median weight was 290 g (range, 265 to 319 g). Ten days prior to the study, frogs were individually weighed, and passive integrated transponder tags (MiniHPT8 8mm FDX-B High Performance PIT Tag; BioMark) were implanted SC in the right thigh. No grossly visible tissue damage or adverse effects were noted at the insertion site of any frog prior to the study. Passive integrated transponder tags were selected over visible implant elastomer tags based on a study[18] reporting migration of visible implant elastomer material to the kidneys in marine toads. All frogs were deemed clinically healthy based on examination by a veterinarian, and no frogs were receiving any medications at the time of the study.

A single frog was acquired with abnormal tissue overlying the cornea of the left eye. Ultrasound and examination by a board-certified veterinary ophthalmologist revealed no evidence of ongoing inflammation in the anterior chamber and an otherwise unremarkable cornea. The lesion appeared separate from the eyelids and third eyelid. The etiology of this lesion is unknown, but both eyes remained static throughout the study period, and the frog was in an otherwise appropriate body condition with a normal physical examination; enrollment in the study was elected.

Frogs were group housed in a single 284-L tank with shallow water, a sponge filter, air stones, and plastic hides for 3 weeks prior to the study. The population was maintained on a 12-hour photoperiod and fed once daily using a pelleted diet provided by their supplier. Frogs were not offered food during the 24-hour study window. Fresh water was obtained from a reservoir of city tap water treated with sodium thiosulfate. Water temperature was allowed to equilibrate with the temperature of the climate-controlled facility (21 to 22 °C). Water quality was assessed daily, and 100% water changes were performed as needed.

Drug administration and sample collection

On the study day, frogs were manually removed from the group tank and placed into individual clear plastic containers (36 X 31 X 33 cm) lined with a moist towel. Frogs were briefly manually restrained in sternal recumbency, and the dorsum of each frog was gently dabbed dry with soft gauze. Transdermal flunixin meglumine (Banamine Transdermal; Merck Animal Health) at 3.3 mg/kg was then administered topically to the central dorsum using a 2-to-20-µL micropipette (Denville Scientific). Three frogs required doses just above 20 µL (21 µL); these frogs were administered 11 µL followed immediately by 10 µL in the same location. A dose of 3.3 mg/kg was selected as this is the labeled dose for cattle[12,13] and the dose used in a prior study in marine toads.[11]

Following drug administration, all frogs were returned to their individual containers for 4 hours and subsequently returned to group housing, where they had access to shallow water above the level of the dorsum, for the remainder of the study period.

A sparse-sampling strategy and population pharmacokinetic modeling were used to minimize the frequency of blood sampling.[11,19,20] Individual frogs were randomly assigned to 2 of the 6 following venipuncture time points: 1, 2, 4, 8, 12, and 24 hours following flunixin administration; as such, 7 frogs were sampled per time point. For venipuncture, frogs were manually restrained with an encircling grip around the pelvic girdle, with care not to touch the site of drug administration on the dorsum. Blood (0.3 to 0.4 mL) was collected from the right (first sample) and left (second sample) popliteal sinus using a 28-gauge needle and attached syringe **(Figure 1)**.[11,21] If, based on visual inspection, a lymphatic fluid–contaminated sample was obtained, the sample was discarded, and the attempt was repeated with a new syringe until blood without visually apparent hemodilution was collected. The total volume of blood collection did not exceed 0.5% of body weight for each frog. Blood was immediately transferred to lithium heparin blood tubes (1.3 mL; Sarstedt), placed on ice, and, within 1 hour of collection, centrifuged at 1,000 X *g* for 10 minutes. Plasma was separated, transferred to cryovials on ice, and frozen at −80 °C within 2 hours of collection.

Figure 1—Popliteal sinus venipuncture technique in an American bullfrog (*Lithobates catesbeianus*). The restrainer holds with an encircling grip just cranial to the pelvis. The phlebotomist gently grasps and extends the distal hindlimb and directs the needle toward the stifle at a 45° angle using a caudomedial approach while maintaining negative pressure.[21]

Frogs were serially monitored at each venipuncture time point for adverse reactions systemically or at the flunixin administration site. Each frog was individually manually restrained for a brief physical examination at 2, 3, and 7 days after flunixin administration; physical examinations included an assessment of posture, demeanor, and integument, both systemically and at the flunixin administration site, and an assessment for the presence of any abnormal clinical signs or behaviors.

Drug analysis

Plasma samples were individually analyzed by UPLC-MS-MS using a previously validated method adhering to the International Council for Harmonization validation guidelines for pharmaceutical analytical methods.[11] The UPLC-MS-MS system consisted of an Acquity UPLC I Class Binary Solvent Manager, an Acquity UPLC Sample Manager Flow-Through Needle, and a Xevo TQD Tandem Mass Spectrometer (Waters Corp). The injection volume was 5 µL for all samples. The column temperature was 35 °C, the sample temperature was ambient, and the run time was 5 minutes.

The UPLC-MS-MS technique was validated using blank plasma from the study population. Drug standards (Sigma Aldrich) for flunixin were > 99% purity. Corrections for the meglumine salt in flunixin were made prior to the development of drug standards. Briefly, a total of 3 replicates at multiple concentrations of flunixin (0.001, 0.005, 0.05, and 0.5 µg/mL) were performed on the same batch on the same day. The intraday precision and accuracy were calculated. The precision was 7.19% to 8.84%, and recovery was 84% to 127%. Validation standards were prepared over a linear range and were used to construct calibration curves, with concentrations ranging from 0.0005 µg/mL to 10 µg/mL. The limit of detection and limit of quantification were recognized as 0.001 µg/mL and 0.005 µg/mL, respectively, based on interday precision and accuracy, signal-to-noise ratio, and shape of the chromatograph.

For drug analysis, plasma samples were thawed and processed using solid-phase extraction, and 50 µL of each sample was combined with 250 µL of 4% phosphoric acid in ultrapure water. Samples were vortexed and then transferred to an Oasis Prime HLB 1-mL cartridge (Waters Corp) and processed with the nitrogen-positive pressure manifold (Biotage). Each sample was washed with 1 mL of 5:95 methanol:ultra-pure water and eluted with 0.5 mL of 60:40 acetonitrile:ultra-pure water and 0.1% formic acid. Samples were then filtered through 0.2-µm polytetrafluoroethylene Whatman Mini-UniPrep syringe filter devices (Cytiva) and analyzed by UPLC-MS-MS utilizing a BEH Phenyl 1.7-µm, 2.1 X 100-mm column (Waters Corp) to quantify flunixin concentrations. 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.

Data analysis

The pharmacokinetic analysis of drug concentration-versus-time profiles was performed with Phoenix WinNonlin software (version 8.0; Certara). A noncompartmental analysis of sparse samples (2 samples per frog; n = 21 frogs) was used to derive the slope of the terminal phase and the half-life ($t_{1/2}$). The area under the plasma concentration-versus-time curve from time 0 to infinity was calculated by the linear trapezoidal rule. The volume of distribution (per fraction absorbed) and clearance (per fraction absorbed; mL/h/kg) were also determined; the values of the volume of distribution and clearance are reported as per fraction absorbed because the IV route was not investigated. The values for C_{max} and time to C_{max} (t_{max}) were extracted directly from the concentration-versus time-curves. Descriptive statistics (mean) are presented for each pharmacokinetic parameter and include all frogs as well as male and female subsets.

Results

Transdermal flunixin meglumine was administered without issue to all 21 frogs, and the administered volumes ranged from 16 to 21 µL (0.016 to 0.021 mL). All blood samples were successfully collected, and flunixin was quantifiable in all samples. A semilogarithmic plot of flunixin concentrations detected in each plasma sample with a line plot of the mean values at each time point is presented in **Figure 2**. Calculated pharmacokinetic parameters from the noncompartmental analysis are presented in **Table 1**. Data related to the spread of plasma flunixin concentrations across the 7 sampled frogs at each time point are conveyed in **Table 2**.

No mortality occurred, and no changes in behavior, appetite, attitude, or activity were observed on the study day or over the 7 days following drug application. At visual inspection at the 1-, 2-, and 4-hour venipuncture time points, a subset of frogs had transparent residue at the site of drug administration. At visual inspection at the 8-, 12-, and 24-hour venipuncture time points (ie, once the frogs had been returned to the group tank with shallow water), residue was no longer visually apparent, and, instead, 18 of 21 frogs had mucus and epithelial loss at the drug application site **(Figure 3)**. Starting at 48 hours after drug administration, 6 of these frogs developed mild erythema and suspected angiogenesis at the drug application site **(Figure 4)**. At the 7-day assessment,

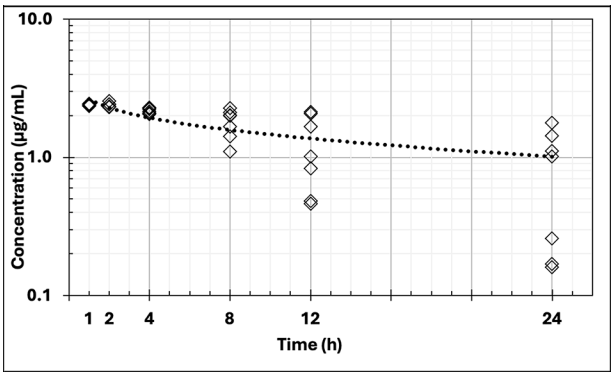

Figure 2—Semilogarithmic plot of individual plasma flunixin concentrations at each time point following administration of flunixin transdermal formulation (3.3 mg/kg) in American bullfrogs (*Lithobates catesbeianus*; n = 21). Each frog was randomly sampled at 2 time points (1, 2, 4, 8, 12, or 24 hours), and the line plot represents the mean value for the 7 samples collected at each time point.

Table 1—Pharmacokinetic parameters from a noncompartmental analysis using plasma data from American bullfrogs (*Lithobates catesbeianus*; n = 21) administered a single 3.3 mg/kg dose of flunixin transdermal formulation.

Parameter	Units	Male	Female	All
λ_z	h^{-1}	0.03	0.13	0.05
$t_{1/2\lambda z}$	h	25.2	5.2	15.0
$AUC_{0\rightarrow\infty}$	h·µg/mL	90.38	52.21	52.94
$\%AUC_{extrap}$	Percentage	53.73	4.91	34.50
CL/F	mL/h/kg	36.51	109.88	62.33
V_d/F	mL/kg	1,328.46	827.4	1,344.73
MRT	h	37.40	8.84	22.03
t_{max}	h	1	1	1
C_{max}	µg/mL	2.41	2.39	2.39

Values reported are the mean for males (n = 9), females (n = 12), and all frogs.

λ_z = Rate constant associated with the terminal elimination phase. $AUC_{0\rightarrow\infty}$ = Total area under the plasma concentration-versus-time curve from time 0 to infinity. $\%AUC_{extrap}$ = Area under the curve extrapolated as a percentage of the total. CL/F = Total body clearance. C_{max} = Maximum plasma concentration. MRT = Mean residence time. $t_{1/2\lambda z}$ = Half-life of λ_z. t_{max} = Time to C_{max}. V_d/F = Volume of distribution.

Table 2—Minimum, maximum, and range of plasma flunixin concentrations following administration of flunixin transdermal formulation (3.3 mg/kg) in American bullfrogs (*Lithobates catesbeianus*; n = 21).

Time point (h)	Minimum (µg/mL)	Maximum (µg/mL)	Range (µg/mL)	2–2.6 µg/mL (frogs)	1–2 µg/mL (frogs)	0.5–1 µg/mL (frogs)	< 0.5 µg/mL (frogs)
1	2.35	2.45	0.10	7	0	0	0
2	2.32	2.55	0.23	7	0	0	0
4	2.06	2.29	0.23	7	0	0	0
8	1.11	2.27	1.16	4	3	0	0
12	0.46	2.13	1.67	2	2	1	2
24	0.16	1.79	1.63	0	4	0	3

Each frog was randomly sampled at 2 of 6 time points over 24 hours, resulting in 7 frogs sampled/time point. The number of frogs within the listed plasma flunixin concentration range is included.

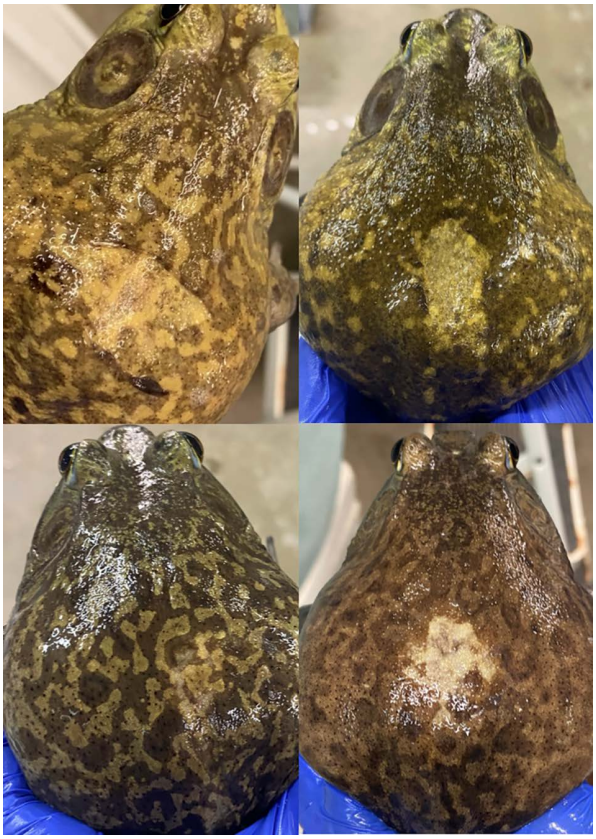

Figure 3—Mucus and/or epithelial loss at the site of drug application (central dorsum) in 18 of 21 American bullfrogs (*Lithobates catesbeianus*) 8, 12, or 24 hours after application of flunixin transdermal formulation at 3.3 mg/kg.

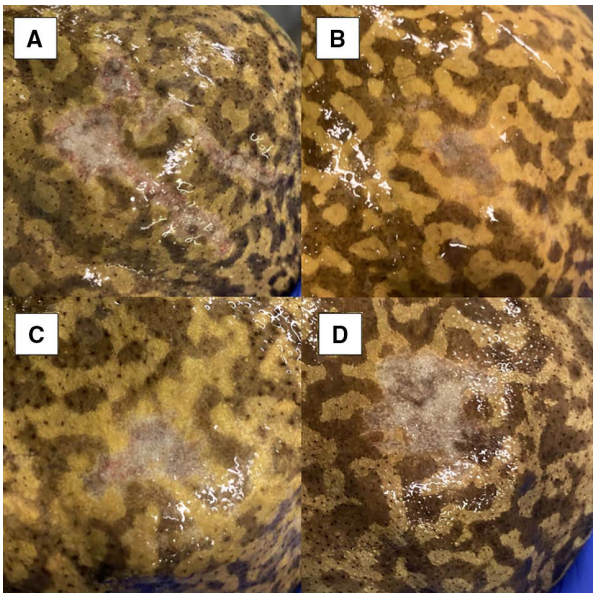

Figure 4—Erythema and suspected angiogenesis noted at the site of drug application (central dorsum) in 6 of 21 American bullfrogs (*Lithobates catesbeianus*) 48 hours after flunixin transdermal formulation application at 3.3 mg/kg. Image A is the frog with subjectively the most overt lesion; in the remaining 5 frogs, lesions were subtle and difficult to capture in a photograph (Images B, C, and D).

the aforementioned lesions were still present but subjectively reduced in 18 of 18 frogs with resolution of erythema. Three of 21 frogs, with sampling time points of 1 and 8 hours, 8 and 24 hours, and 12 and 24 hours, had no changes at the flunixin administration site at all time points, including the 2-, 3-, and 7-day assessments. All 3 of these frogs were male.

Discussion

Transdermal flunixin meglumine at 3.3 mg/kg applied topically to the dorsum of American bullfrogs was systemically absorbed and detected in the plasma in all frogs. In a pooled analysis, the mean C_{max} was 2.39 µg/mL, and this was maintained above 2.00 µg/mL (83.3% of mean C_{max}) for all sampled frogs for at least 4 hours. In a comparable study[11] in marine toads, transdermal flunixin meglumine (3.3 mg/kg) resulted in a C_{max} approximately 3-fold higher (6.31 µg/mL). Furthermore, as the current study did not capture the absorption phase, it should be noted that the actual C_{max} in bullfrogs may be higher than reported. While the minimum plasma concentrations of flunixin necessary for anti-inflammatory and analgesic effects in amphibians are unknown, in other taxa (ie, mammals), significantly lower mean plasma concentrations have been associated with analgesic effects. In healthy donkeys and goats administered transdermal flunixin meglumine at 3.3 mg/kg, a more than 10–fold lower mean C_{max} resulted in a significant reduction in eicosanoid concentrations for up to 96 hours (C_{max}, 0.161 µg/mL in donkeys)[22] and produced an approximately 50% reduction in plasma prostaglandin E2 concentrations (C_{max}, 0.13 µg/mL in goats).[23] Additionally, multiple studies[12,13,24] in dairy cattle (adults and calves) administered transdermal flunixin meglumine (3.3 mg/kg) documented a C_{max} 2-fold lower than the current study (1.08 to 1.17 µg/mL) and a significant improvement in experimentally induced lameness in adult cattle. Thus, while a therapeutic plasma concentration threshold for amphibians has not been determined, it is reasonable to hypothesize that transdermal flunixin meglumine at the studied dose could produce analgesia in bullfrogs and that lower doses might also be effective. While not a direct comparison due to differences in formulation, dosing, species, and route of administration, it should also be noted that 2 pharmacodynamic studies[10,25] in an amphibian species (*Xenopus laevis*) documented analgesic effects from injectable flunixin meglumine (25 mg/kg) administered into the dorsal lymph sac, further supporting the clinical utility of this drug in amphibians.

In the current study, the mean t_{max} of flunixin was 1 hour and ranged from 1 to 2 hours overall. When applied topically, transdermal drugs must diffuse through the epidermis and any extraepidermal layers before being available for systemic absorption in the vascularized dermis.[26] Furthermore, true frogs, including bullfrogs, possess mucus glands in the dorsal epidermis that secrete a lipid-containing mucus layer that covers the epidermis; in contrast, true toads lack these glands.[14,16,17,27] Accordingly, studies[16,17,27] comparing percutaneous absorption of

various drugs between true frogs (*Litoria caerulea*) and true toads (*R marina*) determined that although permeability along the ventrum was similar between species, permeability over the dorsum was consistently higher in marine toads; this highlights the potential influence of mucus in delaying or limiting drug absorption. Although the aforementioned studies would suggest otherwise, the t_{max} in studied bullfrogs was similar to marine toads administered transdermal flunixin meglumine (3.3 mg/kg), which had a reported mean t_{max} of 1 hour. While this may support a similar rate of initial absorption in both species, it should be noted that in both studies the absorption phase was not captured. Thus, in either or both studies, the t_{max} may have been faster than reported as the earliest samples were obtained 1 hour after drug administration. Nonetheless, the current data set supports that despite a mucus layer, absorption of transdermal flunixin meglumine in bullfrogs is clinically rapid and may be a viable alternative route of administration compared to injection.

Following a rapid initial absorption, plasma flunixin concentrations in the current study were maintained above 2.00 µg/mL (83.3% of mean C_{max}) for all sampled frogs at 1, 2, and 4 hours and subsequently for 4 of 7 and 2 of 7 frogs at 8 and 12 hours, respectively. These data support a prolonged (ie, several hour) absorption period in bullfrogs with plasma concentrations maintained close to C_{max} for an extended period of time and are likely an example of flip-flop pharmacokinetics, in which the rate of drug absorption is slower than the rate of drug elimination from the body. In light of the lower C_{max} documented in bullfrogs (2.39 µg/mL) compared to marine toads (6.39 µg/mL), one hypothesis for this finding is that the lipid-rich mucus layer of frogs may have served as a flunixin depot, both permitting continued absorption over an extended period of time and limiting the C_{max} achieved.

Furthermore, while plasma flunixin concentrations were consistent among all bullfrogs sampled at 1, 2, and 4 hours, these concentrations varied considerably at later time points, with a more than 10-fold difference observed between 2 frogs sampled at the 24-hour time point (0.16 vs 1.79 µg/mL, or 6.7% of C_{max} vs 74.6% of C_{max}, respectively). Recall that frogs were returned to shallow water within group housing following sampling at 4 hours; thus, a drug-rich mucus depot could have theoretically been disrupted by physical contact with either splashed water or conspecifics, and this could have contributed to the observed variability in plasma flunixin concentrations beyond 4 hours. Additionally, it is possible that the "lower" concentrations represented true pharmacokinetics and that the "higher" concentrations were a result of additional flunixin meglumine uptake from physical contact with conspecifics, although investigators believe this is less likely. Access to water and conspecifics was provided at 4 hours in the current study to mimic a clinical setting and resume standard-of-care husbandry practices; however, limiting these factors in future studies could help delineate their influence on study results.

Calculated plasma elimination $t_{1/2}$ in bullfrogs in the current study was 15.0 hours, which is longer than other studied species,[12,13,22–24] including a 5-fold longer duration than marine toads (2.79 hours).[11] A myriad of potential differences could explain this disparity between bullfrogs and marine toads, including composition, thickness, vascularization, and permeability of the integument, exocrine secretions, body composition, drug distribution, metabolism, and excretion, and/or relative enzyme abundance.[6,27] Particularly important to note is that the aforementioned mucus may have slowed absorption and/or acted as an extracorporeal depot of flunixin meglumine and thus may have alone or in combination with the aforementioned factors contributed to the identified elimination half-life. It should be noted that in the current study, the extrapolated percentage of the total area under the curve (%AUC_{extrap}) was 34.5%. For the calculated total area under the plasma concentration-versus-time curve from time 0 to infinity to be considered reliable, the %AUC_{extrap} value should ideally be < 20%. As such, a longer sampling period with or without repeated dosing studies is likely needed to determine appropriate dosing intervals. That said, the observed plasma elimination half-life in bullfrogs in the current study would likely support a longer, more clinically practical dosing interval (eg, once daily). Further, while the small sample size of each sex at each time point precluded statistical comparison and introduces the possibility of a type II error, %AUC_{extrap} and $t_{1/2}$ were considerably higher in male frogs (53.73%; 25.2 hours) than in female frogs (4.91%; 5.2 hours), and mean plasma concentrations at 24 hours were higher in males (1.33 µg/mL; n = 3) than in females (0.20 µg/mL; n = 3). Notably, this time point (24 hours) included 2 male frogs with the highest individual flunixin concentrations (1.79 µg/mL and 1.43 µg/mL), both of which were among the 3 frogs (all male) that did not develop any skin lesions during the study. While the influence of sex on this finding is still unknown, it lends further support to the previously described mucus depot hypothesis. As noted, a larger sample size would permit a more thorough evaluation of sex-based differences.

A single dose of transdermal flunixin meglumine in the current study resulted in no mortality or observable systemic adverse effects; however, most frogs developed mucus and epithelial loss, with a smaller subset experiencing subsequent erythema and suspected angiogenesis at the flunixin application site. Amphibian skin is involved in a multitude of vital processes, including respiration, osmoregulation, and pathogen defense, and serves as a physical barrier to environmental insults.[6,28] Thus, while the observed cutaneous lesions did not result in any clinical signs of adverse effects, the short- and long-term consequences are not known and may present additional considerations for a less-hardy species and/or clinically ill frogs. Additionally, the effects of repeated dosing on cutaneous integrity remain unknown.

Transdermal flunixin meglumine is delivered in a viscous, somewhat adherent propylene glycol–based vehicle with red dye. Each milliliter of Banamine

Transdermal pour-on contains 50 mg flunixin (equivalent to 83 mg flunixin meglumine), 150 mg pyrrolidone, 50 mg L-menthol, 500 mg propylene glycol dicaprylate/dicaprate NF (national formulary), 0.20 mg FD&C Red 40, and glycerol monocaprylate NF qs (quantity sufficient). Transparent, adherent residue was noted at the flunixin administration site on some frogs prior to their return to water, and subsequent physical disruption of this residue may have contributed to the observed lesions. A control group consisting of topical application of the vehicle alone was not used in the current study; however, the addition of this test group and/or investigation of the topical application of flunixin meglumine formulated for injection could help tease out the contribution of the drug's vehicle to the observed lesions. In marine toads, transdermal flunixin formulation at the same dose (3.3 mg/kg) was well tolerated and did not result in significant gross or histologic cutaneous or visceral lesions attributable to flunixin meglumine administration, supporting species differences.[11]

Limitations of the current study include a lack of pharmacodynamic assessment, the small sample size, the necessity of a sparse-sampling protocol and population-based pharmacokinetic approach, and the absence of bioavailability data. Further assessment of safety using additional diagnostics (eg, blood biochemistry, euthanasia, and histopathology) was beyond the scope of this study. Finally, in clinical settings, repeated doses of NSAIDs are commonly clinically indicated; however, the pharmacokinetics and provisional safety of multiple doses of transdermal flunixin meglumine were not evaluated in the present study. Banamine Transdermal is FDA approved only for use in cattle and was used extralabel in the present laboratory study. In clinical practice, use of this product in anurans must comply with provisions of AMDUCA and 21 Code of Federal Regulations §530.

In conclusion, transdermal flunixin meglumine applied topically to the dorsum at 3.3 mg/kg was systemically absorbed in American bullfrogs, reached C_{max} values between 1 and 2 hours, and was detected in plasma at 24 hours. Plasma flunixin concentrations were similar across individuals at 1, 2, and 4 hours but increased in variability at 8, 12, and 24 hours. Mean C_{max} was lower and elimination half-life was longer in American bullfrogs compared to a toad species (*R marina*) administered the same dose of transdermal flunixin meglumine in a previous study.[11] Cutaneous lesions (mucus loss and epithelial loss) occurred in most frogs at the administration site, but no behavior change, mortality, or other adverse clinical effects were observed. Transdermal flunixin meglumine at 3.3 mg/kg administered topically to American bullfrogs was absorbed in a clinically appropriate time frame, and plasma concentrations exceeded established therapeutic ranges in other species; however, variability in plasma concentrations over time and topical side effects may limit its use.

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