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SHORT COMMUNICATION

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Effect of rain on absorption after transdermal application of flunixin in calves

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Abstract

Flunixin is a nonsteroidal anti-inflammatory drug (NSAID) that has anti-inflammatory, anti-pyretic, and analgesic effects. Recently, a novel transdermal formulation was developed (Finadyne® Transdermal, MSD Animal Health) and is now the first NSAID registered to be administered as a pour-on product in cattle. According to the manufacturer's instructions, the pour-on product should be applied only to dry skin and exposure to rain should be avoided for at least 6 hr after application. The objective of the study was to evaluate the effect of simulated exposure to light or heavy rain on flunixin absorption and bioavailability within the first 4 hr after administration. Therefore, an isocratic HPLC method was developed to quantify flunixin concentrations in bovine serum by UV detection. Light rain decreased flunixin absorption only when rain started immediately after flunixin administration, while light rain starting more than 30 min after administration of flunixin had no effect on absorption. Absorption and bioavailability of flunixin was impacted under simulated heavy rain conditions, when exposure to rain occurred within one hour after the application of the pour-on formulation, but not later.

KEYWORDS

absorption, calf, flunixin, rain, transdermal

1 | INTRODUCTION

Finadyne® Transdermal (MSD Animal Health) is licensed for the reduction in pyrexia associated with bovine respiratory disease or acute mastitis and for the reduction in pain and lameness. The drug is labeled to be applied on dry skin and exposure to rain must be prevented for at least 6 hr. However, in some situations when housing is not available, moisture due to rain may influence the absorption of the drug.

To investigate the effect of rain within 4 hr after administration, cattle were exposed to simulated light or heavy rain, initiated either immediately or at various time points after administration of the product. The hypothesis of the study was that the absorption of flunixin is decreased if it is raining when the drug is applied or if the rain starts shortly after application. The effect of exposure to rain on absorption of flunixin was expected to be minimal if the rainfall started at least 60 min after application. In addition, intensity of rain was expected to influence the absorption of flunixin.

Prior to initiation of the study, to comply with animal welfare and protection legislation, the study protocol was approved by two institutional and governmental animal protection committees (GZ68.205/0136-WF/V/3b/2015).

2 | MATERIAL AND METHODS

The study was conducted on nine male calves older than 14 d of age. The drug was administered according to label (3.33 mg/kg bodyweight) to the calves as a pour-on at the midline of the back.

The application of the drug was conducted under three simulated rain conditions:

1. Dry conditions (control)
2. Light rain (<2.5 mm/hr)
3. Heavy rain (>7.6 mm/hr)

All calves were exposed to the following nine experimental conditions:

1. Control dry conditions (no exposure to rain)
2. Light rain initiated immediately after treatment and continued for 30 min
3. Light rain initiated 30 min after treatment and continued for 30 min
4. Light rain initiated 60 min after treatment and continued for 30 min
5. Light rain initiated 240 min after treatment and continued for 30 min
6. Heavy rain initiated immediately after treatment and continued for 30 min
7. Heavy rain initiated 30 min after treatment and continued for 30 min
8. Heavy rain initiated 60 min after treatment and continued for 30 min
9. Heavy rain initiated 240 min after treatment and continued for 30 min

The experimental conditions were applied in a different order for each calf. The study was designed as a cross-over study, allocating the treatments following a 9 × 9 Latin square design. A 4-day washout period was provided between treatments. The rain was simulated using an irrigating system (Micro Rain Systems e.K.) adapted from greenhouse irrigation. Immediately after finishing the simulated rain, the calves were dried using a professional hair dryer for animals (Pet Dryer CFJ002, Go Pet Club LLC).

For blood collection, the area for the incision was anaesthetized (0.3 ml Procamidor *s.c.*, 20 mg/ml procaine hydrochloride; Richter Pharma AG). An intravenous catheter (Cavafix® Certo® with Splittocan® 338; catheder 1.1 × 1.7 mm/16G, 32 cm length; cannula 1.8 × 2.35 mm/14G, length 8 cm; B. Braun Melsungen AG) was placed in the jugular vein. Nine blood samples (5 ml) were taken per trial (before treatment at 0 min, 15 min, 30 min, 60 min, 120 min, 240 min, 8 hr, 12 hr, and 24 hr after application). Blood was collected from the catheter using a syringe and placed into serum tubes (Vacuette Tube 9 ml *Z* Serum Clot Activator; Greiner Bio-One GmbH). The blood samples were centrifuged at 1,500 × *g* for 10 min. The serum was placed into microcentrifuge tubes (Safe-Lock Tubes 2.0 ml, Eppendorf-AG) and stored at −21°C until analysis. Flunixin meglumine concentrations were measured using HPLC at the laboratory of the Medical University Vienna, Institute for Pharmacology. The laboratory was blinded for treatment regimens of the calves. The analyses were performed as repeated determination in two samples. Samples were prepared using 25 µl standard (clonixin), 50 µl 2 M NaH2PO4, tert. Butyl-methyl ether was used for extraction. The supernatant was vaporized and transferred on 100 µl HPLC medium. HPLC was performed using VWR Hitachi (Chromaster Series) as isocratic analysis (C18 column, velocity 1.00 ml/min at 30°C). Calibration was conducted using cattle serum and flunixin. Flunixin was detected using UV light at 245 nm.

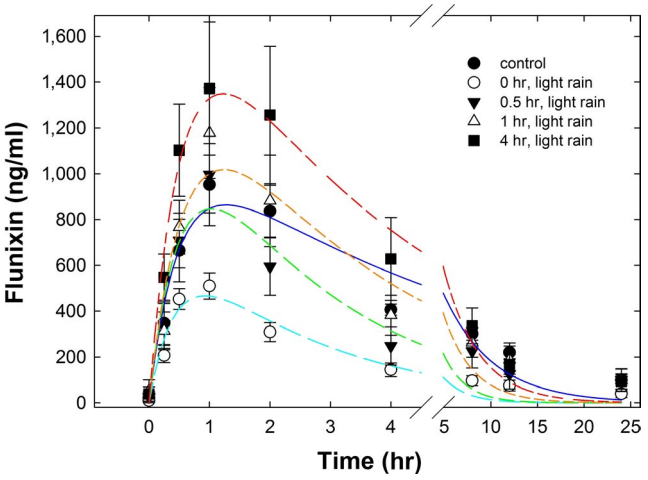

FIGURE 1 Arithmetic mean ± standard deviation of the concentration against time curves of the nine calves in the study when a simulated light rain (0.25 mm/6 min) was applied continuously for 30 min after administration of 3.33 mg/kg flunixin meglumine as a pour-on administration (solid circle = no rain control; empty circle = rain started at 0 min after administration; solid triangle = rain started at 30 min after administration; empty triangle = rain started at 60 min after administration; solid square = rain started at 240 min after administration) [Colour figure can be viewed at wileyonlinelibrary.com]

Concentration–time curves were created from the data applying linear trapezoidal rule calculation. From the curves, Cmax, Tmax, and the area under the curve (AUC) were calculated.

A mixed-effects linear model, (rain regime × treatment period × interaction of rain regime by treatment period) which allowed for accounting of the repeated measurements and provided the effects of treatment period, sequence of treatments, and carryover effects, was used. The animals were considered a random factor to provide intra- and inter-animal variability. Statistical significance was set at a level of $p < .05$.

3 | RESULTS

Figures 1 and 2 summarize the concentrations as arithmetic mean and *SD* separately for light and heavy rain which was started at

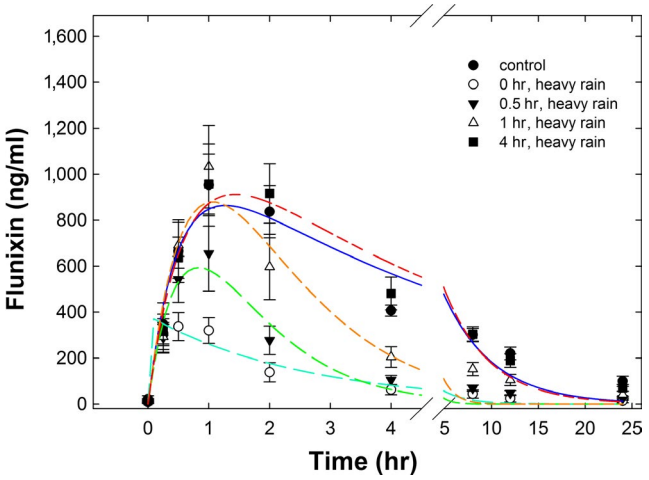

FIGURE 2 Arithmetic mean ± standard deviation of the concentration against time curves of the nine calves in the study when a simulated heavy rain (0.76 mm/6 min) was applied continuously for 30 min after administration of 3.33 mg/kg flunixin meglumine as a pour-on administration (solid circle = no rain control; empty circle = rain started at 0 min after administration; solid triangle = rain started at 30 min after administration; empty triangle = rain started at 60 min after administration; solid square = rain started at 240 min after administration) [Colour figure can be viewed at wileyonlinelibrary.com]

different time points following administration of flunixin transdermal. Although there were differences among calves, the concentration–time curves followed the Bateman function. C_{max} was reached earlier in comparison with control ($p < .05$) when light rain started at t0 and heavy rain started at t0 and t30 (Table 1). T_{max}, similar to C_{max}, was also influenced by the volume of the rain and length of time between administration of the flunixin transdermal and the onset of the rain. Calves exposed to light rain at t0 and those exposed to heavy rain starting at 0, 30, and 60 min showed shorter T_{max} in comparison with control ($p < .05$).

The AUC was decreased ($p < .05$) in comparison with control if light rain was initiated at 0 min or if heavy rain was initiated at 0, 30, and 60 min after flunixin application (Table 1).

4 | DISCUSSION

Flunixin meglumine is a potent nonselective inhibitor of cyclooxygenase (Lees, Giraudel, Landoni, & Toutain, 2004). Recently, a transdermal formulation has been developed It is recommended to be applied only to dry skin and exposure to moisture must be prevented for at least 6 hr after application.

The first clinical study on the pharmacokinetics of transdermal flunixin meglumine in Holstein calves was published by Kleinhenz et al. (2016). Thiry, Fournier, Roy, and Catala (2017) evaluated the effect of flunixin transdermal pour-on solution on prostaglandin E_2 (PGE_2) synthesis in bovine inflammatory exudate after induction of inflammation in cattle. The effects of transdermal flunixin meglumine on pain biomarkers and the impact of pain on the pharmacokinetics of the pour-on formulation administered at the time of cautery disbudding in calves were described by Kleinhenz et al. (2017), Kleinhenz, Van Engen, et al. (2018b). The pharmacokinetics of multiple doses of transdermal flunixin meglumine administered to adult Holstein dairy cows every 24 hr for a total of three doses were published by Kleinhenz, Gorden, et al., 2018.

To your knowledge, the effect of wet conditions or rain on the pharmacokinetics has not yet been described. In the present study, exposure to simulated rain after transdermal application reduced the absorption and bioavailability of flunixin. Light rain resulted in decreased absorption only when rain started immediately after application. On the contrary, light rain which started later (≥30 min after application) had no effect on absorption.

The effect of heavy rain reduced the absorption and bioavailability of flunixin if rain started within one hour after application. When heavy rain started 4 hr after application, it did not affect C_{max}, T_{max}, or AUC.

TABLE 1 Numerical values (arithmetic means) of C_{max}, T_{max}, and AUC

Rain treatment		C_{max} ± *SD* (ng/ml)		T_{max} ± *SD* (h)		AUC ± *SD* (ng/ml × h)	
Intensity of rain	Time[a]						
No rain	No rain	997.89	477.26	2.00	2.44	7,087.11	1,296.38
Light rain[b]	0	540.73*	161.30	*0.83	0.25	2,725.29*	1,385.26
	30	1,004.13	492.87	1.06	0.39	5,853.45	4,653.57
	60	1,177.97	593.77	1.11	0.33	7,011.47	5,246.16
	240	1,415.34	882.16	1.28	0.56	8,596.21	5,585.78
Heavy rain[c]	0	410.88*	230.91	0.63*	0.28	1,294.30*	1,145.42
	30	697.81	489.85	0.83*	0.25	2,300.73*	1,023.22
	60	1,033.20	539.29	1.00*	0.00	4,311.38*	2,336.09
	240	1,041.41	372.72	1.55	1.01	6,999.03	1986.22

[a]Time (min) after administration of flunixin meglumine pour-on.

[b]0.25 mm/6 min.

[c]0.76 mm/6 min.

*indicates significant difference to control ($p < .05$).

Therefore, the manufacturer's requirement to prevent rain exposure for at least 6 hr after administration of the product provides assurance for reliable drug absorption. The practical conclusion of the study is that the exposure to light rain should be avoided during at least 30 min during light rain and 4 hr under heavy rain after administration of the drug.

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CONFLICT OF INTEREST

ST is employed at Merck Animal Health, and CG is employed at Intervet GesmbH.

AUTHOR CONTRIBUTIONS

All listed authors qualify for authorship according to the criteria given in the JVPT Editorial Policies and Ethical Considerations. TW, ST, and CG had the idea for the study. BAM, AK, and CS performed the clinical study and took the blood samples in the calves. MW, KK, and MF made the pharmacological analyses and developed the method for rapid determination of flunixin in blood. BAM and MW wrote the manuscript. TW, ST, and CG made critical revisions to the manuscript. All authors have read and approved the final manuscript.

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