Effect of age on the pharmacokinetics and pharmacodynamics of flunixin meglumine following intravenous and transdermal administration to Holstein calves

Michael D. Kleinhenz DVM
Nicholas K. Van Engen DVM, PhD
Patrick J. Gorden DVM, PhD
Joe S. Smith DVM, MPS
Butch KuKanich DVM, PhD
Suzanne M. Rajewski PhD
Philip Walsh BVSc
Steven Perkins BVSc
Johann F. Coetzee BVSc, PhD

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From the Department of Veterinary Diagnostic and Production Animal Medicine (Kleinhenz, Van Engen, Gorden, Smith, Coetzee) and Pharmacology Analytical Support Team (Rajewski, Coetzee), College of Veterinary Medicine, Iowa State University, Ames, IA 50011; the Department of Anatomy and Physiology and Institute for Computational Comparative Medicine, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506 (KuKanich); Orchard Veterinary Centre, 59 Loughgall Rd, Armagh BT61 7NG, Northern Ireland (Walsh); and Castle Veterinary Surgeons, Montalbo Rd, Barnard Castle, DL12 8ED, England (Perkins). Dr. Kleinhenz’s present address is Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506. Dr. Van Engen’s present address is Johnson Research LLC, 24007 US-20, Parma, ID 83660. Dr. Coetzee’s present address is Department of Anatomy and Physiology and Institute for Computational Comparative Medicine, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506.

Address correspondence to Dr. Coetzee (jcoetzee@vet.k-state.edu).

OBJECTIVE
To determine the effect of age on the pharmacokinetics and pharmacodynamics of flunixin meglumine following IV and transdermal administration to calves.

ANIMALS
8 healthy weaned Holstein bull calves.

PROCEDURES
At 2 months of age, all calves received an injectable solution of flunixin (2.2 mg/kg, IV); then, after a 10-day washout period, calves received a topical formulation of flunixin (3.33 mg/kg, transdermally). Blood samples were collected at predetermined times before and for 48 and 72 hours, respectively, after IV and transdermal administration. At 8 months of age, the experimental protocol was repeated except calves received flunixin by the transdermal route first. Plasma flunixin concentrations were determined by liquid chromatography–tandem mass spectrometry. For each administration route, pharmacokinetic parameters were determined by noncompartmental methods and compared between the 2 ages. Plasma prostaglandin (PG) E2 concentration was determined with an ELISA. The half maximal inhibitory concentration of flunixin on PG_E2 concentration was determined by nonlinear regression.

RESULTS
Following IV administration, the mean half-life, area under the plasma concentration-time curve, and residence time were lower and the mean clearance was higher for calves at 8 months of age than at 2 months of age. Following transdermal administration, the mean maximum plasma drug concentration was lower and the mean absorption time and residence time were higher for calves at 8 months of age than at 2 months of age. The half maximal inhibitory concentration of flunixin on PG_E2 concentration at 8 months of age was significantly higher at 2 months of age. Age was not associated with the percentage change in PG_E2 concentration following IV or transdermal flunixin administration.

CONCLUSIONS AND CLINICAL RELEVANCE
In calves, the clearance of flunixin at 2 months of age was slower than that at 8 months of age following IV administration. Flunixin administration to calves may require age-related adjustments to the dose and dosing interval and an extended withdrawal interval. (Am J Vet Res 2018;79:568–575)

Scientific literature regarding the effects of age on the pharmacokinetics of drugs in cattle and other veterinary species is sparse. Cattle, in particular, undergo changes in body structure and composition as they mature from preruminant calves to adult ruminants. Those changes include alterations in total water composition, body surface area, and adipose tissue development; female ruminants also undergo changes associated with gestation and lactation. Changes in drug absorption, metabolism, and renal excretion as individuals mature are the basis for age-dependent differences in the pharmacokinetics of various drugs. In cattle, the effect of age on the pharmacokinetics of some NSAIDs has been investigated. However, there is a lack of information re-
garding the effect of age on the pharmacokinetics of flunixin meglumine (flunixin) in cattle.

Flunixin is a nicotinic acid derivative and is the only NSAID approved by the FDA for use in cattle in the United States. Injectable formulations of flunixin are labeled for the treatment of pyrexia associated with bovine respiratory disease and endotoxic mastitis and inflammation associated with endotoxemia in adult cattle. Flunixin has also been reported to be useful as an adjunctive treatment for neonatal calves with diarrhea.6

Because flunixin is an NSAID, it has direct effects on COX enzymes associated with inflammation. The COX pathway is responsible for the conversion of arachidonic acid to PGE\textsubscript{2}, PGI\textsubscript{2}, PGF\textsubscript{2\alpha}, and thromboxane. Cyclooxygenase has 2 isoforms, COX-1 and COX-2, and each isoform has distinct functions. The COX-1 isoform is constitutively produced in tissues and is associated with homeostasis. The COX-2 isoform is constitutively expressed in some tissues and can be markedly upregulated subsequent to inflammatory insults, tissue injury, or localized hypotension. Expression of COX-2 products such as PGE\textsubscript{2} and PGI\textsubscript{2} correlates best with the anti-inflammatory function of flunixin and other NSAIDs. For example, inhibition of PGE\textsubscript{2} and PGI\textsubscript{2} is positively correlated with anti-inflammatory activity, and an increase in PGE\textsubscript{2} and PGI\textsubscript{2} concentrations is associated with poor anti-inflammatory activity.

The objective of the study reported here was to investigate the effect of age on the pharmacokinetics and pharmacodynamics of flunixin following IV and transdermal administration to Holstein calves. We hypothesized that the pharmacokinetics and associated pharmacodynamics of flunixin would differ as calves age owing to changes in physiology, body composition, and growth.

**Materials and Methods**

**Animals and study design**
All study procedures were reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC Nos. 6-15-8039-B and 5-15-8016-B). The study was conducted in 2 periods with a 6-month interval between the 2 periods, and the same calves were used for both periods. Eight 6-week-old Holstein bull calves were obtained from a single source before initiation of period 1. The calves were weaned and had a mean ± SD weight of 60.2 ± 7.3 kg. The calves were acclimated for 14 days prior to initiation of period 1, during which they were accustomed to being restrained with a halter and lead rope and were drenched with amprolium\textsuperscript{4} (10 mg/kg, PO) once daily for 5 consecutive days for coccidiosis control. At the beginning of period 2, the calves were 8 months old and had a mean ± SD weight of 222 ± 7.3 kg.

Each period consisted of IV and transdermal phases and had a crossover design, with a 10-day washout period between the 2 phases. For the first phase of period 1, all calves received flunixin meglumine\textsuperscript{5} (2.2 mg/kg) IV to facilitate determination of the bioavailability of flunixin following transdermal administration. For each calf prior to IV flunixin administration, a catheter was aseptically placed in each jugular vein. The catheter in the left jugular vein was used for flunixin administration, after which it was flushed with heparinized saline (0.9% NaCl with 1% sodium heparin) solution and immediately removed. The catheter in the right jugular vein was used for collection of serial blood samples. From each calf, a blood sample (approx 20 mL) was collected immediately before and at 3, 6, 10, 15, 30, 45, and 60 minutes and 2, 4, 6, 8, 12, 24, 36, and 48 hours after flunixin administration. Each blood sample was collected into a syringe and then immediately placed into two 10-mL blood collection tubes\textsuperscript{2} that contained sodium heparin as an anticoagulant. The tubes were gently inverted several times to ensure that the blood was adequately mixed with the anticoagulant. The catheter was flushed with heparinized saline solution after collection of each blood sample and was removed immediately after collection of the last blood sample for phase 1. All blood samples were placed on ice immediately after collection and transported to the laboratory for processing within 1 hour after collection. At the laboratory, all blood samples were centrifuged at 1,500 X g for 10 minutes. The plasma was harvested from each blood sample, placed in a cryovial, and stored frozen at –80°C until analyzed.

During phase 2 of period 1, each calf was administered flunixin meglumine\textsuperscript{4} (3.33 mg/kg) transdermally. The flunixin formulation used was approved for transdermal administration to cattle in the European Union and Canada and was administered with a single-use syringe in accordance with the label directions. For each calf, the drug was applied to the skin of the dorsal topline beginning between the scapulas and extending to the tail head. Prior to drug administration, a catheter was aseptically placed in a jugular vein of each calf for collection of serial blood samples. From each calf, a blood sample was collected via the jugular catheter as described for phase 1 immediately before and at 10, 20, 30, 40, 50, 60, and 90 minutes and 2, 4, 6, 8, 12, 24, 36, 48, 56, and 72 hours after flunixin administration. The catheter was removed immediately after collection of the last blood sample for phase 2.

Period 2 was initiated 6 months after completion of phase 2 of period 1, and the 8 calves used for period 1 were used for period 2. The experimental protocol for period 2 was identical to that described for period 1, except the administration route order was reversed. During phase 1 of period 2, all calves received flunixin meglumine\textsuperscript{4} (3.33 mg/kg) transdermally, and blood samples were collected immediately before and at 30, 60, and 90 minutes and 2, 4, 6, 8, 12, 24, 48, and 72 hours after drug administration. During phase 2 of period 2, all calves received flunixin meglumine\textsuperscript{4}.
(2.2 mg/kg) IV, and blood samples were collected immediately before and at 3, 6, 10, 15, 30, 45, and 60 minutes and 2, 4, 6, 8, 12, 24, 36, and 48 hours after drug administration.

**Plasma flunixin meglumine determination**

The flunixin concentration in each plasma sample was determined by use of high-performance liquid chromatography–tandem mass spectroscopy as described. Briefly, the mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Separation was achieved with a C18 column. Three ions for flunixin were measured in negative ion mode at m/z 295 → 251, 210, and 197, with a retention time of 5.60 minutes. Only the 251 ion was used for quantification.

The standard curve for flunixin in bovine plasma had a quadratic shape and ranged from 0.005 to 10 µg/mL. Samples with flunixin concentrations > 10 µg/mL were diluted with blank bovine plasma (ie, plasma from cattle that were not treated with flunixin). The curve was accepted when the correlation coefficient exceeded 0.99 and the measured values were within 15% of the expected values. The accuracy and coefficient of variation for flunixin were 108% and 7.3%, respectively. The limit of quantification for flunixin was 0.005 µg/mL, and the limit of detection was 0.003 µg/mL.

**Pharmacokinetic analysis**

Pharmacokinetic analysis was performed by use of noncompartmental methods with computer software. Individual animal pharmacokinetic values were calculated, and descriptive statistics (geometric mean and median and range) were reported as described. For each period, the bioavailability of flunixin following transdermal administration was calculated as follows: (AUCtransdermal/IV dose)/AUCIV dose.

**Determination of plasma PGE2 concentration**

Ex vivo plasma PGE2 concentrations were determined by use of methods adapted from Fraccaro et al and Stock et al. Prior to centrifugation, 2-mL aliquots of anticoagulated blood were reserved from blood samples collected before and at 1, 2, 4, 12, 24, and 48 hours after IV flunixin administration and from blood samples collected before and at 1, 2, 4, 12, 24, 48, 72 hours after drug administration during the transdermal phase of each period for determination of plasma PGE2 concentration. With the exception of aliquots reserved from blood samples collected before flunixin meglumine administration (ie, baseline samples), each 2-mL aliquot was spiked with lipopolysaccharide from *Escherichia coli* O111:B4 (10 µg/mL). Baseline samples were used as controls and were spiked with a volume of PBS solution equal to the volume of lipopolysaccharide added to each of the other 2-mL aliquots. All samples were incubated for 24 hours at 37°C, after which they were centrifuged at 400 Xg for 10 minutes. Plasma was harvested from each sample and pipetted into individual cryovials. The cryovials were placed on dry ice to freeze the plasma and then stored at −80°C until analyzed.

To determine PGE2 concentration, plasma proteins were precipitated with methanol at a 1:5 dilution. Then, samples were centrifuged at 3,000 Xg for 10 minutes, and the supernatant was decanted and used for analysis. The PGE2 concentration in each supernatant sample was determined by use of a commercially available ELISA. The coefficient of variation for intra-assay variability was 8.2%, and interassay variability was 11.2%. For each calf during each phase of each period, the IC50 of flunixin on PGE2 concentration was determined by mathematical regression analysis. For each sample relative to that for the baseline sample for the phase and period being evaluated was calculated as follows: (sample PGE2 − baseline PGE2)/baseline PGE2 X 100.

**Statistical analysis**

The respective data distributions for all pharmacokinetic parameters were assessed for normality by means of the Shapiro-Wilk test. Comparisons between the 2 age groups were performed with unpaired t tests for parametric parameters and Wilcoxon rank sum tests for nonparametric parameters. One calf had a negative MAT during the transdermal phase (phase 1) of period 2, and MAT data from that calf were excluded from that comparison between the 2 ages.

For each route of flunixin administration, a mixed linear regression model was used to assess the effect of age (2 vs 8 months), duration between flunixin meglumine administration and blood sample collection (time), and interaction between age and time on the percentage change in plasma PGE2 concentration; a random effect for calf was included in the model to control for repeated measures within individual calves. The IC50 of flunixin on plasma PGE2 concentration was compared between the 2 age groups by use of a Wilcoxon rank sum test, and the difference in the IC50 between the 2 age groups at each time point was evaluated with a paired t test. All analyses were performed with statistical software, and values of P ≤ 0.05 were considered significant.

**Results**

**Flunixin pharmacokinetics**

No adverse effects associated with flunixin administration were observed in any of the calves during periods 1 and 2. The plasma flunixin concentrations over time after IV and transdermal adminis-
The pharmacokinetic parameters for flunixin following IV and transdermal administration for both ages were also summarized (Table 1). When flunixin

![Graph](image)

**Figure 1**—Mean ± SEM plasma flunixin concentrations over time following administration of an injectable solution of the drug at a dose of 2.2 mg/kg IV (A) or a topical formulation of the drug at a dose of 3.33 mg/kg transdermally (B) to 8 healthy weaned Holstein bull calves at 2 months (dashed line) and 8 months (solid line) of age. At 2 months of age, all calves received the injectable solution of flunixin; then, after a 10-day washout period, calves received the topical formulation of flunixin. Blood samples were collected at predetermined times before and for 48 and 72 hours, respectively, after IV and transdermal drug administration. At 8 months of age, the experimental protocol was repeated except all calves received flunixin by the transdermal route first.

### Table 1—Pharmacokinetic parameters determined for flunixin meglumine following IV and transdermal administration to 8 healthy weaned Holstein bull calves at 2 and 8 months of age.

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Parameter</th>
<th>2 months of age</th>
<th>8 months of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean (range)</td>
<td>Geometric mean (range)</td>
<td>P value*</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–∞&lt;/sub&gt; (h•µg/mL)</td>
<td>14.76 (10.17 to 23.04)</td>
<td>9.00 (6.52–10.39)</td>
<td>0.002</td>
</tr>
<tr>
<td>C0 (µg/mL)</td>
<td>21.24 (15.80 to 30.87)</td>
<td>20.72 (16.50–32.05)</td>
<td>0.875</td>
</tr>
<tr>
<td>CL (mL/min/kg)</td>
<td>2.48 (1.59 to 3.61)</td>
<td>4.08 (3.53–5.62)</td>
<td>0.002</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>5.44 (4.37 to 7.61)</td>
<td>3.45 (2.68–4.00)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>λ&lt;sub&gt;z&lt;/sub&gt; (1/h)</td>
<td>0.13 (0.09 to 0.16)</td>
<td>0.20 (0.17–0.26)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MRT&lt;sub&gt;0–∞&lt;/sub&gt; (h)</td>
<td>4.56 (3.67 to 7.24)</td>
<td>2.53 (1.88–3.52)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>V&lt;sub&gt;s&lt;/sub&gt; (L/kg)</td>
<td>0.68 (0.56 to 0.84)</td>
<td>0.62 (0.50–0.89)</td>
<td>0.277</td>
</tr>
<tr>
<td>V&lt;sub&gt;z&lt;/sub&gt; (L/kg)</td>
<td>1.17 (1.92 to 1.58)</td>
<td>1.22 (1.05–1.38)</td>
<td>0.801</td>
</tr>
<tr>
<td>Transdermal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–∞&lt;/sub&gt; (h•µg/mL)</td>
<td>8.99 (4.60 to 14.04)</td>
<td>8.52 (4.03–11.5)</td>
<td>0.683</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</td>
<td>0.96 (0.48 to 1.68)</td>
<td>0.54 (0.33–1.20)</td>
<td>0.021</td>
</tr>
<tr>
<td>CL/f (mL/min/kg)</td>
<td>6.18 (3.96 to 12.07)</td>
<td>6.52 (4.83–13.76)</td>
<td>0.820</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>9.31 (5.9 to 25.31)</td>
<td>13.24 (6.10–28.80)</td>
<td>0.189</td>
</tr>
<tr>
<td>λ&lt;sub&gt;z&lt;/sub&gt; (1/h)</td>
<td>0.07 (0.03 to 0.12)</td>
<td>0.05 (0.02–0.11)</td>
<td>0.188</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>1.89 (1.00 to 4.00)</td>
<td>2.14 (1.00–4.00)</td>
<td>0.513</td>
</tr>
<tr>
<td>MAT (h)</td>
<td>3.07 (2.81–14.98)</td>
<td>12.94 (6.11–21.53)</td>
<td>0.004</td>
</tr>
<tr>
<td>MRT&lt;sub&gt;0–∞&lt;/sub&gt; (h)</td>
<td>9.10 (6.76 to 14.90)</td>
<td>15.74 (9.05–24.36)</td>
<td>0.006</td>
</tr>
<tr>
<td>V&lt;sub&gt;f&lt;/sub&gt; (L/kg)</td>
<td>4.98 (2.55 to 13.94)</td>
<td>7.47 (2.77–21.69)</td>
<td>0.243</td>
</tr>
<tr>
<td>F</td>
<td>0.40 (0.23 to 0.61)</td>
<td>0.63 (0.31–1.17)</td>
<td>0.199</td>
</tr>
</tbody>
</table>

The study was conducted in 2 periods with a 6-month interval between the 2 periods, and the same calves were used for both periods. Each period consisted of 2 phases, with a 10-day washout period between the 2 phases. At 2 months of age (period 1), all calves received flunixin meglumine (2.2 mg/kg, IV) during the first phase and flunixin meglumine (3.33 mg/kg, transdermally) during the second phase. Blood samples were collected at predetermined times before and for 48 and 72 hours, respectively, after IV and transdermal drug administration. At 8 months of age (period 2), the experimental protocol was repeated except all calves received flunixin by the transdermal route first. Data were not normally distributed for AUC<sub>0–∞</sub>, CL, t<sub>1/2</sub>, and λ<sub>z</sub> when the drug was administered by the IV route or for t<sub>1/2</sub> when the drug was administered by the transdermal route.

*Comparisons between the 2 age groups were performed with unpaired t tests for parametric parameters and Wilcoxon Rank sum tests for nonparametric parameters. †One calf had a negative MAT and was excluded from the analysis for that parameter.

λ<sub>z</sub> = Terminal rate constant. AUC<sub>0–∞</sub> = Area under the plasma concentration-time curve from time 0 extrapolated to infinity. C0 = Plasma concentration extrapolated to time 0. CL/f = Clearance per fraction of the dose absorbed. F = Bioavailability. MRT<sub>0–∞</sub> = Mean residence time from time 0 extrapolated to infinity. t<sub>max</sub> = Time of maximum plasma concentration. V<sub>f</sub> = Volume of distribution per fraction of the dose absorbed.
was administered IV, the median AUC ($P = 0.002$) and $t_{1/2}$ ($P = 0.001$) and geometric mean MRT ($P < 0.001$) were significantly lower, whereas the median CL ($P = 0.002$) and terminal rate constant ($P < 0.001$) were significantly greater for calves at 8 months of age, compared with the corresponding values at 2 months of age. When flunixin was administered transdermally, the geometric mean $C_{\text{max}}$ was significantly ($P = 0.021$) lower, whereas the geometric mean MAT ($P = 0.004$) and MRT ($P = 0.006$) were significantly greater for calves at 8 months of age, compared with the corresponding values at 2 months of age.

**Percentage change in PGE$_2$ concentration**

The percentage change in PGE$_2$ concentration over time for blood samples collected after IV and transdermal flunixin administration to calves at both ages was plotted (Figure 2). When flunixin was administered by the IV route, the percentage change in PGE$_2$ concentration was significantly ($P < 0.001$) associated with the duration between drug administration and blood sample collection (time) but was not associated with age ($P = 0.172$) or the interaction between age and time ($P = 0.228$). Likewise, when flunixin was administered by the transdermal route, the percentage change in PGE$_2$ concentration was significantly ($P = 0.001$) associated with time but was not significantly associated with age ($P = 0.090$) or the interaction between age and time ($P = 0.706$). The mean ± SEM IC$_{50}$ of flunixin on plasma PGE$_2$ concentration for 2-month-old calves (10.8 ± 5.9 ng/mL) was significantly ($P = 0.026$) lower than that for 8-month-old calves (37.7 ± 11.7 ng/mL).

**Discussion**

Results of the present study indicated that the pharmacokinetics of flunixin following both IV and transdermal administration to calves varied with age. Interestingly, more pharmacokinetic parameters had significant differences between calves at 2 months of age and 8 months of age when flunixin was administered by the IV route than when it was administered by the transdermal route. Following IV administration, the plasma CL of flunixin was slower for calves at 2 months of age than at 8 months of age, which resulted in the AUC, $t_{1/2}$, and MRT of flunixin being greater for calves at 2 months of age than at 8 months of age. Those differences were not observed when flunixin was administered by the transdermal route.

The pharmacokinetics of flunixin following IV and transdermal administration to calves at 2 months of age was similar to that reported for similarly aged calves in another study$^7$ conducted by our research group. The MRT and MAT for flunixin following transdermal administration to the 2-month-old calves of that study$^7$ were similar to those for the 2-month-old calves and substantially lower than those for the 8-month-old calves of the present study. Thus, the findings of the present study appear to be supported by the results of that study.$^7$

In another study$^{11}$ in which the pharmacokinetics and tissue depletion of flunixin following IV administration to 3- to 6-week-old calves were described, the $V_{ss}$ (0.63 L/kg) was similar to the $V_{ss}$ (0.68 L/kg) for the 2-month-old calves of the present study, but the MRT (12.54 hours) and $t_{1/2}$ (12.88 hours) were greater than twice the mean MRT (4.56 hours) and $t_{1/2}$ (5.44 hours) for the 2-month-old calves of this study. The authors of that report$^{11}$ attributed the fairly long MRT and $t_{1/2}$ to the fact that the CL of drugs in calves is generally slower than that in adult cattle. Following IV administration of flunixin in the present study, the mean CL (4.08 mL/min/kg) of the drug for calves at 8 months of age was 1.6 times the mean CL (2.48 mL/min/kg) of the drug at 2 months of age, whereas the mean MRT (2.53 hours) and $t_{1/2}$ (3.45 hours) of flunixin for calves at 8 months of age were significantly shorter than the corresponding values 2 months of age. Collectively, these findings support

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**Figure 2**—Mean ± SEM percentage change in PGE$_2$ concentration over time following IV (A) and transdermal (B) administration of flunixin meglumine to 8 calves at 2 months (dashed line) and 8 months (solid line) of age as described in Figure 1. For each blood sample collected following flunixin meglumine administration, the percentage change in plasma PGE$_2$ concentration was calculated as follows: (sample PGE$_2$ – baseline PGE$_2$)/baseline PGE$_2$ X 100, where baseline PGE$_2$ was the concentration of PGE$_2$ in the plasma of the blood sample collected immediately before drug administration. Notice the scale of the y-axis differs between the 2 panels. See Figure 1 for remainder of key.

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$^2$See Figure 1 for remainder of key.
an age-dependent effect on the pharmacokinetics of flunixin following IV administration to cattle and provide compelling evidence of the need to extend the slaughter withdrawal interval from the labeled slaughter withdrawal time when the drug is administered in an extralabel manner to calves, especially those intended for veal production.

The effect of age on the pharmacokinetics of other NSAIDs has been described in other studies. However, unlike the present study, those studies did not use the same animals for all experimental phases. When phenylbutazone was administered IV, the CL was slower and the MRT and t1/2 were longer for young calves relative to older calves. Following IV administration of either the R-(−) or S-(+) enantiomer of ketoprofen, the AUC and MRT of both enantiomers were greater and the CL of the S-(+) enantiomer was slower for calves, compared with the corresponding values for adult cattle. Similar age-dependent pharmacokinetic changes have been described following IV administration of carprofen to calves. Following IV administration of the stereoselective drug flurbiprofen, the CL was slower and the AUC was lower for calves than for adult cattle; however, the MRT and t1/2 of flurbiprofen did not differ significantly between the calves and adult cattle of that study. Thus, the effect of age on the pharmacokinetics of NSAIDs following administration to cattle appears to vary, and a clear generalization of age-dependent effects on the pharmacokinetics of NSAIDs as a drug class cannot be provided.

Results of a study on the effect of age on the pharmacokinetics of flunixin following IV administration to horses suggest that age is negatively correlated with CL and positively correlated with t1/2. Those findings differ from those obtained for the calves of the present study following IV administration of flunixin. However, as with other NSAIDs, age did not appear to have a significant effect on the Vₐ of flunixin for the horses of that study or the calves of this study.

The effect of age on the pharmacokinetics of various antimicrobials following administration to various mammals has been reviewed. For calves specifically, the author of that review reported that the plasma elimination half-life of antimicrobials tends to decrease as age increases. Another study indicated that the CL of ceftiofur, an antimicrobial that, like flunixin, is highly protein bound, is significantly slower and the AUC is significantly greater for 1-day-old calves than for 6-month-old calves.

In the present study, age was not significantly associated with the percentage change in PGE₂ in 8-month-old calves, and IV administration of the drug resulted in a smaller negative percentage change in PGE₂ concentration in calves at 8 months than that at 2 months of age. This suggested that suppression of PGE₂ synthesis was reduced when flunixin was administered to the calves at 8 months of age relative to that when the drug was administered to the calves at 2 months of age. The percentage change in PGE₂ concentration mirrored the plasma flunixin concentration and associated pharmacokinetic parameters in the present study. The difference in the percentage change in PGE₂ concentration between the 2 ages was attributed to the slower CL and longer t½ of flunixin and lower IC₅₀ of flunixin on PGE₂ concentration for calves at 2 months relative to those at 8 months of age. On the basis of the ex vivo PGE₂ suppression results, flunixin administered IV at the high end of the label dose (2.2 mg/kg) should produce anti-inflammatory effects for 24 hours in calves ≤ 2 months old, and a dosing interval of 24 hours should be sufficient for such calves.

The differences between the 2 age groups in the percentage change in PGE₂ concentration following transdermal administration of flunixin likely reflected age-related effects on pharmacokinetic and pharmacodynamic parameters. The significant differences in the percentage change in PGE₂ concentration (ie, flunixin-induced anti-inflammatory effects) between the 2 ages over time were attributed to the lower Cmax, longer MRT, and greater IC₅₀ for 8-month-old calves relative to the corresponding values for 2-month-old calves. Consequently, the clinically perceived anti-inflammatory effects of flunixin for calves at 8 months of age may be less than those at 2 months of age, but further research in which specific clinically relevant endpoints are evaluated is warranted to validate that supposition.

The fact that most of the pharmacokinetic parameters for flunixin did not differ significantly following transdermal administration to calves at 2 months of age and again at 8 months of age might be attributable to skin thickness. The skin of cattle thickens as animals age; thus, the skin of 8-month-old calves is thicker than the skin of 2-month-old calves. Thicker skin typically results in slower drug absorption (ie, longer MAT), a lower Cmax, and a longer t½ following transdermal drug administration. Those pharmacokinetic changes are indicative of flip-flop kinetics, in which drug absorption becomes the rate-limiting step in drug elimination. However, for the calves of the present study, the skin thickness difference between calves at 2 and 8 months of age was not sufficient to significantly affect the CL of flunixin following transdermal administration.

Other significant differences in pharmacokinetic parameters observed between the 2 ages evaluated in the present study might be attributed to age-related differences in body composition and development. As calves mature, the amount of body water decreases...
es as muscle mass and adipose tissue increase.\(^1\) Results of multiple studies\(^2\*-5\) indicate that age-related changes in body composition affect the MRT, \(t_{1/2}\), and CL of various drugs. Additionally, hepatic metabolism changes as cattle mature, and the hepatic clearance of drugs in young calves is generally slower than that in older cattle.\(^2\) Those principles held true for the calves of the present study, as evidenced by the fact that the CL increased and \(t_{1/2}\) decreased following IV administration of flunixin to calves at 8 months of age relative to those at 2 months.

Published literature regarding the IC\(_{50}\) of flunixin on the plasma PGE\(_2\) concentration of cattle is sparse, and evaluation of the effect of age on the IC\(_{50}\) of flunixin on PGE\(_2\) concentration is lacking. The primary goal of the studies\(^15,16\) that have evaluated IC\(_{50}\) data for cattle was to determine the COX-1 or COX-2 preference and to compare different NSAID formulations. On the basis of the IC\(_{50}\) results of the present study, further research is necessary to elucidate the anti-inflammatory mechanism of flunixin.

The design of the present study was unique in that the same calves were used for both experimental periods. The phases in each period were blocked such that there was only 1 treatment in each block to avoid a period effect in the final analysis. Moreover, by blocking the treatments, the washout period could be standardized to reduce the possibility of carryover flunixin between phases. Although we recognize that not having both treatments represented in all study phases might be a source of bias, we feel that such bias was unlikely given the short time (10 days) between the 2 phases of each period.

When the present study was conducted, the transdermal formulation of flunixin meglumine was approved for use in cattle in the European Union and Canada. After the study was completed, the US FDA approved that formulation for the treatment of fever associated with bovine respiratory disease and the control of pain associated with foot rot in cattle.

In the present study, multiple significant age-related differences were identified for the pharmacokinetic parameters of flunixin following IV and transdermal administration to calves at 2 and 8 months of age. The differences in pharmacokinetics following IV administration of flunixin reflected changes in the CL of the drug from the body as calves mature. However, age was not associated with flunixin-induced PGE\(_2\) inhibition, which suggested that age-related changes in the pharmacokinetics of a drug might not be reflected as alterations in clinically evident outcomes. Following transdermal flunixin administration, significant age-related differences were observed only for MAT and MRT. Age was not significantly associated with the overall percentage change in PGE\(_2\) concentration, but the percentage change in PGE\(_2\) concentration was greater for 2-month-old calves than for 8-month-old calves during the first 48 hours after transdermal flunixin administration. Thus, results of the present study suggested that flunixin administration to young calves may require age-related dose adjustments and an extended withdrawal interval. Additionally, age-related differences in the affinity of flunixin for COX enzymes require further investigation.

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### Footnotes

1. a. Corid, Merial LLC, Duluth, Ga.
   b. Banamine injectable solution, Merck Animal Health, Madison, NJ.
   c. BD Vacutainer, Franklin Lakes, NJ.
   e. Phenomenex, Torrance, Calif.
   f. Phoenix 64, Certara, Princeton, NJ.
   g. Sigma-Aldrich Corp, St Louis, Mo.
   h. Cayman Chemicals, Ann Arbor, Mich.
   i. Prism7, GraphPad Software Inc, La Jolla, Calif.
   j. JMP Pro, version 12.0, SAS Institute Inc, Cary, NC.

### References


