Comparison of pharmacokinetics and milk elimination of flunixin in healthy cows and cows with mastitis

Lindsey W. Kissell, PhD; Teresa L. Leavens, PhD; Ronald E. Baynes, DVM, PhD; Jim E. Riviere, DVM, PhD; Geof W. Smith, DVM, PhD

Objective—To determine whether pharmacokinetics and milk elimination of flunixin and 5-hydroxy flunixin differed between healthy and mastitic cows.

Design—Prospective controlled clinical trial.

Animals—20 lactating Holstein cows.

Procedures—Cows with mastitis and matched control cows received flunixin IV, ceftiofur IM, and cephapirin or ceftiofur, intramammary. Blood samples were collected before (time 0) and 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 36 hours after flunixin administration. Composite milk samples were collected at 0, 2, 12, 24, 36, 48, 60, 72, 84, and 96 hours. Plasma and milk samples were analyzed by use of ultra-high-performance liquid chromatography with mass spectrometric detection.

Results—For flunixin in plasma samples, differences in area under the concentration-time curve and clearance were detected between groups. Differences in flunixin and 5-hydroxy flunixin concentrations in milk were detected at various time points. At 36 hours after flunixin administration (milk withdrawal time), 8 cows with mastitis had 5-hydroxy flunixin concentrations higher than the tolerance limit (ie, residues). Flunixin residues persisted in milk up to 60 hours after administration in 3 of 10 mastitic cows.

Conclusions and Clinical Relevance—Pharmacokinetics and elimination of flunixin and 5-hydroxy flunixin in milk differed between mastitic and healthy cows, resulting in violative residues. This may partially explain the high number of flunixin residues reported in beef and dairy cattle. This study also raised questions as to whether healthy animals should be used when determining withdrawal times for meat and milk. (J Am Vet Med Assoc 2015;246:118–125)

Flunixin is an NSAID approved for use in beef and dairy cattle for the modulation of inflammation in endotoxemia and the control of pyrexia associated with bovine respiratory disease and acute mastitis. When administered in accordance with label instructions, withdrawal time for violative residues in milk is 36 hours. In milk, the marker residue is a metabolite of flunixin, 5-hydroxy flunixin, the tolerance limit of which is 2 ng/mL. Nonsteroidal anti-inflammatory drugs such as flunixin are reportedly the second most prescribed class of drugs by dairy veterinarians and the most frequently administered analgesics in cattle. Because flunixin is the only NSAID approved for use in dairy cattle in the United States and is routinely used on dairy farms, there is a potential for violative residues. Although flunixin is regularly used on dairy farms, milk samples in the United States are not routinely tested for 5-hydroxy flunixin residues. This may be partially a result of information in early reports, which suggested that flunixin residues were not a concern because residues were not detected in milk after IV or IM administration. However, more recent research with more sensitive analytic equipment clearly has revealed that 5-hydroxy flunixin and flunixin accumulate in milk at concentrations well above the tolerance limit.

A recent flunixin surveillance study in which samples were obtained from 500 tanker truck loads of milk
found that 1 of the 500 milk samples had 5-hydroxy flunixin concentrations above the tolerance limit. That study revealed that violations of flunixin residues in milk occur in the dairy industry, although the cause of these residues remains unknown. Possible explanations for violative flunixin residues in milk include not observing the withdrawal time for milk, extralabel use of flunixin (eg, altering the dose, route of administration, frequency, or duration of treatment), and altered milk elimination as a result of a disease process. Investigators of several studies have reported alterations in plasma pharmacokinetics of drugs in diseased animals, compared with results for their healthy counterparts. Investigators of a recent study found a correlation between low milk production and prolonged flunixin elimination in milk. Considering that mastitis often results in a substantial decrease in milk production, violative flunixin residues may be prevalent in milk from mastitic cows.

Since 2005, the USDA Food Safety Inspection Service has reported an increasing number of flunixin residue violations in milk from dairy cattle. This increase in the number of violations attributable to flunixin residues has led to flunixin becoming the second most common residue violation (behind only penicillin) in bulk dairy cattle. These residue violations in meat have primarily been attributed to extralabel use of flunixin. However, delayed plasma clearance caused by a disease process may result in a prolongation of residues in meat and could contribute to the high number of flunixin violations in edible tissues. Therefore, the objective of the study reported here was to determine whether plasma pharmacokinetics and elimination of flunixin and 5-hydroxy flunixin in milk would differ between healthy cows and cows with clinical mastitis.

Materials and Methods

Animals—Twenty lactating Holstein cows from a dairy farm in North Carolina were used in a prospective controlled clinical trial. Cows weighed between 545 and 676 kg (1,199 and 1,487 lb). Ten cows were identified as having naturally occurring mastitis as defined by abnormal appearance of milk and a red or swollen mammary gland. Some mastitic cows had systemic signs of other diseases, whereas others did not. Ten healthy cows were then matched to the cows with mastitis on the basis of parity (ie, current lactation number), number of days in lactation, and milk production to serve as control cows. This study was approved by the North Carolina State University Institutional Animal Care and Use Committee.

Mastitis was diagnosed during the morning milking for all cows. Within 2 hours after the diagnosis of clinical mastitis, a catheter was aseptically placed in a jugular vein. Heart rate and rectal temperature were recorded for each cow, and 5 mL of milk was aseptically collected from each mastitic gland for aerobic bacterial culture prior to treatment.

Experimental design—One dose of flunixin meglumine (2.2 mg/kg [1 mg/lb], IV) was administered to all cows in the jugular vein contralateral to the vein in which the catheter had been placed. All cows also received cefiofur (2.2 mg/kg, IM, q 24 h for 3 days). For intramammary antimicrobial treatment, cows were allocated into 2 groups (groups 1 and 2), each of which consisted of 10 cows (5 cows with mastitis and 5 paired control cows). Mastitic cows of group 1 received cepahpin (1 syringe [10 mL], intramammary, q 12 h for 3 days) in the affected gland, whereas mastitic cows of group 2 received cefiofur (1 syringe [10 mL], intramammary, q 24 h for 5 days) in the affected gland. The healthy cows of group 1 received intramammary treatments of cepahpin administered in the same gland as their matched cows with mastitis, and healthy cows of group 2 received intramammary treatments of ceftiofur administered in the corresponding gland. For example, if the mastitic cow was affected in the right rear mammary gland, the matched control cow also was given the same intramammary antimicrobial in the right rear mammary gland.

Collection of blood and milk samples—Blood samples were collected from the jugular catheter into heparinized tubes before flunixin administration (time 0) and 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 36 hours after administration. Blood samples were centrifuged at 1,690 X g for 10 minutes at 4°C; plasma was harvested and frozen at −20°C until analysis of flunixin and metabolite concentrations. Prior to flunixin administration, 5 mL of foremilk was manually collected from each gland of every cow; the 4 gland samples were pooled to provide a baseline composite sample for each cow. Additional composite milk samples were collected with a sampling device during milking. These composite milk samples were collected 2, 12, 24, 36, 48, 72, 84, and 96 hours after flunixin administration. All sample collection times, except for the sample collected at 2 hours, were times that corresponded with the farm’s regular milking schedule. Milk samples were immediately frozen at −20°C until analysis.

Analysis of milk and plasma samples—Flunixin and 5-hydroxy flunixin concentrations were quantified in both plasma and milk by use of ultra-high-performance liquid chromatography with mass spectrometric detection. Plasma samples were extracted. Samples were thawed, and 0.3 mL of plasma was combined with 0.9 mL of 0.5% citric acid in acetonitrile. Samples were mixed in a sonicator for 5 minutes and then centrifuged for 10 minutes at 3,300 X g. The supernatant was loaded on a solid-phase extraction cartridge. Elute from the cartridge was collected, placed in a 55°C evaporator, dried under a stream of nitrogen, reconstituted in 300 μL of mobile phase, and filtered through a 0.22-μm nylon syringe filter. Injection volume was 5 μL. Concentrations were derived by comparing peak areas for the samples with those for an external standard curve made from spiked plasma samples analyzed via the same procedures. Milk samples were also extracted. Samples (0.5 mL of milk) and 1.5 mL of 0.5% citric acid in acetonitrile were combined in a centrifuge tube, and the same procedures were used as those described for extraction and quantification of plasma samples. The ultra–high-performance liquid chromatography with a mass spectrometric detection system consisted of a high-strength silica T3 column (1.8 μm inside diameter, 2.1 X 100 mm) and filter disk. The mobile phase was a mixture of acetonitrile and 0.1% acetic acid in water (68:32 [vol/vol]). A single quadrupole mass spectrometer was used in positive electrospray ionization mode. Ions with m/z of 297.0 and 313.0 were used for quantification of flunixin and 5-hydroxy flunixin, respectively. Column

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temperature was 30°C, and sample temperature was 4°C. Run times were 2.2 minutes. The LOQ was determined as 10 times the SD of 6 blank samples. The LOD was determined as 3 times the SD of 6 blank samples. The LOD and LOQ for flunixin and 5-hydroxy flunixin in plasma were 10 and 20 ng/mL, respectively, and the linear range was 20 to 30,000 ng/mL. The LOD and LOQ for flunixin and 5-hydroxy flunixin in milk were 1 and 2 ng/mL, respectively, and the linear range for milk was 2 to 1,000 ng/mL. Relative SD for both inter- and intraday accuracy was <15% at all concentrations ($r^2 > 0.99$).

Pharmacokinetic analysis—A non-compartmental analysis of flunixin plasma concentration versus time profiles was performed with pharmacokinetic modeling software. The AUC$_{\text{ss}}$ and AUMC were calculated by use of the linear trapezoidal rule; AUC$_{\text{0-}}$ was performed with pharmacokinetic model- compartmental analysis of flunixin plasma concentration versus time profiles was performed with the aid of commercially available software. All values were expressed as mean ± SD. Flunixin plasma pharmacokinetic parameters and flunixin and 5-hydroxy flunixin concentrations in milk were compared by means of Satterthwaite approximation for degrees of freedom. Values of $P < 0.05$ were considered significant.

Results

Cows—Ten cows with clinical mastitis were identified and enrolled in the study between May and August 2012; the 10 healthy control cows were enrolled during the same time period (Table 1). Mean heart rate of the mastitic cows was significantly ($P = 0.01$) different from that of the control cows; however, no difference

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control cows</th>
<th>Mastitic cows</th>
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<tbody>
<tr>
<td>Age of cow (y)</td>
<td>4.8 ± 1.5</td>
<td>4.9 ± 1.8</td>
</tr>
<tr>
<td>Current lactation No.</td>
<td>2.8 ± 0.9</td>
<td>3.0 ± 1.5</td>
</tr>
<tr>
<td>No. of days in lactation</td>
<td>153 ± 73</td>
<td>142 ± 74</td>
</tr>
<tr>
<td>Milk production prior to study (kg/d [lb/d])</td>
<td>33.4 ± 3.8 (73.5 ± 8.4)</td>
<td>29.9 ± 4.3 (65.8 ± 9.5)</td>
</tr>
<tr>
<td>305-day mature equivalent milk production (kg [lb])</td>
<td>10,152 ± 2,030 (22,335 ± 4,466)</td>
<td>8,155 ± 1,860 (17,940 ± 4,092)</td>
</tr>
<tr>
<td>Rectal temperature (°F [°C])</td>
<td>38.33 ± 3.81 (100.0 ± 6.7)</td>
<td>38.99 ± 0.99 (102.0 ± 1.8)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>74.2 ± 10.4*</td>
<td>93.6 ± 10.7</td>
</tr>
</tbody>
</table>

*Value differs significantly ($P = 0.01$) from the value for the mastitic cows.
in rectal temperature was evident between the 2 groups (P = 0.1). Results of bacteriologic culture of milk obtained from 8 of the 10 mastitic cows indicated *Klebsiella pneumoniae* infection, and the remaining 2 cows had *Escherichia coli* infection.

**Plasma**—Mean ± SD peak plasma concentration after flunixin administration was 18.35 ± 5.40 µg/mL and 24.45 ± 8.22 µg/mL for control cows and cows with mastitis, respectively; these values did not differ significantly (P = 0.06). Flunixin was detectable in the plasma of cows with mastitis for up to 24 hours after administration, which was 12 hours more than flunixin was detectable in the plasma of healthy cows (Figure 1). Clearance for the cows with mastitis was significantly (P = 0.01) reduced, compared with clearance for the healthy cows (Table 2). The AUC<sub>0-∞</sub> for the mastitic cows was more than twice the AUC<sub>0-∞</sub> for the control cows and was significantly (P = 0.004) different between the 2 groups. Plasma terminal elimination half-life (%P = 0.9), V<sub>darea</sub> (%P = 0.1), and V<sub>ds</sub> (%P = 0.09) did not differ significantly between the healthy and mastitic cows.

**Milk**—Residues of 5-hydroxy flunixin above the tolerance limit were detected in milk samples from 8 of the mastitic cows at the labeled withdrawal time (36 hours after administration of flunixin) and were still detectable in milk samples from 3 mastitic cows up to 48 hours after administration (Table 3). However, no 5-hydroxy flunixin residues above the tolerance limit were detected in milk of the control cows > 24 hours after flunixin administration (Figure 2). Milk concentrations of 5-hydroxy flunixin were significantly different between the control cows and cows with mastitis for samples obtained at both 2 and 12 hours, with the mastitic cows having lower 5-hydroxy flunixin concentrations in milk at both time points.

Flunixin residues in milk persisted for up to 60 hours after administration in some of the mastitic cows; in contrast, flunixin residues were detectable in milk of control cows for only up to 24 hours after administration (Figure 2). Flunixin concentrations in milk were significantly different between groups at all time points, with the mastitic cows having substantially greater flunixin concentrations in milk, compared with flunixin concentrations in milk of control cows (Table 4).

**Discussion**

The objective of the present study was to determine whether plasma pharmacokinetics and elimination of flunixin and 5-hydroxy flunixin in milk of lactating dairy cattle would differ between cows with naturally occurring mastitis and paired control cows without mastitis. The significant difference in AUC<sub>0-∞</sub> between the 2 groups was a result of a higher maximum plasma concentration and measureable concentrations of flunixin in the plasma for up to 24 hours after administration to mastitic cows. Clearance calculated for healthy cows in the present study was similar to findings reported in the literature. Reduced clearance in the mastitic cows may have been attributable to alterations in hepatic drug metabolism. This is similar to results for another NSAID, carprofen, for which clearance is reduced by 40% in mastitic cows, compared with that in healthy control cows. Inflammation and infection can greatly impact drug metabolism and protein binding, which may also contribute to alterations in clearance. In the present study, the mean plasma terminal elimination half-life for flunixin after IV administration corresponded with that found in other studies.

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**Figure 2**—Flunixin (A) and 5-hydroxy flunixin (B) concentrations in milk after administration of flunixin (time 0) to 10 mastitic cows (diamonds and dashed line) and 10 healthy control cows (squares and dotted line). In panel B, notice the tolerance limit for violative residues of 5-hydroxy flunixin in milk (solid horizontal line). Also notice that the scale on the y-axis differs between panels. Within a time point, values with different letters differ significantly (P < 0.05).

**Table 4**—Mean ± SD flunixin concentration in milk (µg/L) obtained after administration of flunixin to 10 control cows and 10 cows with mastitis.

<table>
<thead>
<tr>
<th>Time after flunixin administration (h)</th>
<th>Control cows</th>
<th>Mastitic cows</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4.91 ± 3.32 (n = 6)</td>
<td>224.0 ± 232.2 (n = 10)</td>
<td>0.009</td>
</tr>
<tr>
<td>12</td>
<td>16.77 ± 16.68 (n = 10)</td>
<td>102.8 ± 88.8 (n = 10)</td>
<td>0.010</td>
</tr>
<tr>
<td>24</td>
<td>3.18 ± 0.367 (n = 4)</td>
<td>37.17 ± 36.02 (n = 10)</td>
<td>0.010</td>
</tr>
<tr>
<td>36</td>
<td>&lt; LOQ (n = 10)</td>
<td>26.54 ± 32.01 (n = 7)</td>
<td>—</td>
</tr>
<tr>
<td>48</td>
<td>&lt; LOQ (n = 10)</td>
<td>11.37 ± 15.69 (n = 7)</td>
<td>—</td>
</tr>
<tr>
<td>60</td>
<td>&lt; LOQ (n = 10)</td>
<td>13.02 ± 10.83 (n = 3)</td>
<td>—</td>
</tr>
</tbody>
</table>

See Table 3 for key.
The Vd_{area} and Vd_{ss} were also consistent with values reported in the literature,5,7,23–25,30; however, they were greater than expected for a drug that is highly (99%) protein bound.

The high number of violations for flunixin residues in tissues of dairy cattle may in part be attributable to disease-induced alterations in pharmacokinetics, particularly alterations in clearance of a drug. Investigators in numerous studies6–18 have reported changes in pharmacokinetics as a result of inflammation. In 1 study21 in horses, investigators found significantly greater concentrations of several NSAIDs in inflamed tissues, compared with the concentrations in healthy tissues. Similarly, there is an increased partition of both ceftiofur and erythromycin into infected bovine tissue chambers, compared with the partitions for healthy bovine tissue chambers.32,33 In rabbits, inflammation can increase capillary permeability, thus altering flunixin partitioning into tissues.19 In that same study,19 endotoxemic rabbits also had a significantly lower clearance and longer elimination half-life, compared with values for healthy control rabbits. A recent surveillance study34 found that cows culled because of clinical signs or evidence of disease had a significantly higher incidence of violative tissue flunixin concentrations at slaughter than did healthy-appearing dairy cows. The high number of flunixin tissue residues identified in culled dairy cattle may be related to mastitis-induced alterations in drug clearance.

Milk concentrations of 5-hydroxy flunixin in the control cows at each time point in the present study were similar to milk concentrations in other studies.5,7,30 The persistence of 5-hydroxy flunixin residues in milk beyond the labeled withdrawal time may in part be related to a decrease in milk production as a result of mastitis.19 There are a paucity of reports describing the effect of milk production on drug elimination, especially for systemically administered drugs. However, several studies16–40 have found a correlation between low milk production and prolonged drug elimination for some intramammary drugs. A previous study7 conducted by our laboratory group found that milk production was a significant covariate for the depletion of 5-hydroxy flunixin concentrations in milk over time. In that study,7 cows producing < 20 kg (44 lb) of milk/d eliminated 5-hydroxy flunixin more slowly than did cows producing 30 kg (66 lb) of milk/d. In the present study, all of the mastitic cows produced < 6 kg (13.2 lb) of milk/d during the experimental period. Prior to clinical mastitis diagnosis, these cows were producing a mean ± SD of 29.9 ± 4.3 kg (65.8 ± 9.5 lb) of milk/d. The persistence of 5-hydroxy flunixin residues in milk beyond the labeled withdrawal time may also have been attributable to binding of 5-hydroxy flunixin to inflamed mammary gland tissues. In a study35 conducted to examine the pharmacokinetics and pharmacodynamics of flunixin in calves, investigators found that the drug binds to inflamed tissue and achieves high concentrations in inflammatory exudate. The accumulation of flunixin in inflamed tissue and slow clearance from exudate may partially explain the reason that 5-hydroxy flunixin residues were detectable in milk for up to 60 hours after administration.

Flunixin concentrations in the milk of control cows of the present study were comparable to concentrations reported on the US FDA website.35 The significantly greater concentration of flunixin in the milk from mastitic cows was unexpected. Several factors may have contributed to this finding. One explanation may be disruption of the blood-milk barrier. Both K pneumonae and E coli are known to increase mammary gland vascular permeability and cause breakdown of the blood-milk barrier.41 This may have resulted in leakage of flunixin from the blood into the milk prior to hydroxylation by the liver. Considering that all cases of mastitis in the present study were caused by coliform bacteria, it is unclear whether the increase in flunixin concentrations in milk would be as pronounced with other causes of mastitis (ie, gram-positive organisms). Another potential explanation for the significantly greater flunixin concentrations in milk from mastitic cows may be related to impaired hepatic metabolism as a result of mastitis. Disease states can impair hepatic metabolism of drugs and alter clearance of the parent compound. Similarly, the prolonged persistence of flunixin residues in the milk of mastitic cows (up to 60 hours after administration) may also have been related to the decrease in milk production associated with mastitis as well as flunixin binding to inflamed mammary gland tissue.

Mastitis induces physical and chemical changes in both milk and affected mammary glands; these changes have the potential to alter distribution and elimination of drugs through the mammary gland.38 Inflammation of a mammary gland leads to vascular permeability changes that often enhance systemic absorption and perhaps distribution of drugs into the udder. For example, gentamicin is not detected in plasma following intramammary administration in clinically normal mammary glands but is well absorbed in mammary glands of cows with mastitis.42 Similarly, in a study43 that involved the use of polymyxin B, the drug was not found in the blood or untreated mammary glands following intramammary administration in clinically normal cattle; however, substantial systemic absorption was evident in cows with experimentally induced coliform mastitis. Investigators of a study37 conducted to evaluate the influence of E coli endotoxin–induced mastitis on the pharmacokinetics of the NSAID carprofen found a significant reduction in systemic clearance, prolonged terminal elimination half-life, and increased milcarprofen concentrations in mastitic cows after IV administration, compared with results for healthy control cows. Finally, investigators of a study44 that involved the use of an intramammary preparation of cefoperazone sodium reported significantly greater systemic drug absorption, half-life in the milk, and MRT in cows with subclinical mastitis, compared with results for healthy control cows.

The parenteral administration of ceftiofur to treat clinical mastitis in the present study represented extra-label use of this drug. It was a standard procedure on this farm to administer ceftiofur IM to all cattle that had mastitis and moderate to severe signs of systemic disease (particularly if milk production decreased substantially). Investigators of another study45 found that IM adminis-
tation of ceftiofur in cows with severe coliform mastitis reduced the proportion of cases that resulted in death in the form of culling. Although there has been a prohibition on the extralabel use of cephalosporins in the United States, these drugs can still be used for nonlabeled indications as long as the administration is in accordance with the labeled dose and duration.8 The dose of 2.2 mg/kg, IM, every 24 hours for 3 days is in accordance with the label direction and would not represent illegal use of this drug. Control cows without mastitis also received 3 doses of ceftiofur so that they were treated in the same manner as the mastitic cows. Administration of antimicrobials to one group and not the other could potentially have affected the pharmacokinetics of flunixin. Cepha-
pirin is labeled for intramammary administration every 12 hours for a total of 2 doses; therefore, administration of this drug every 12 hours for 3 days represented extralabel use. The protocol for this farm at the beginning of the study was to administer cepha
pirin via the intramammary route every 12 hours until the milk became visually normal. Because of the need to have a consistent duration of treatment for all cows in the study, farm personnel elected to use intramammary administration of cepha
pirin for 6 total treatments (every 12 hours for 3 days) in all cows regardless of the duration of clinical mastitis. Toward the middle of the study, farm personnel elected to change intramammary treatment to ceftiofur because most cases had been caused by coliform bacteria. Cef
tiofur was administered via the intramammary route once a day for 5 days, which was in accordance with label directions. Treatment of only the mastitic cows with antimicrobials could have potentially resulted in a drug-drug interaction, which may have altered the pharmacokinetics and confounded comparisons between healthy and mastitic cows. Therefore, the same treatment regimen administered to the mastitic cows was also administered to the control cows.

Violative drug residues in milk are a primary concern for the dairy industry. Residues in milk as a result of administration of NSAIDs can result in major economic losses to producers and pose a potential health hazard to consumers. Strict financial penalties and suspension of a producer’s permit to sell milk are possible outcomes of violative drug residues detected in milk. To prevent economic losses to producers, it is imperative that the labeled withdrawal time is accurate for the disease condition in which the drug is administered. Problems arise when there is discord between the labeled withdrawal time, which is calculated on the basis of data from healthy animals, and the time required for diseased animals to eliminate a drug.

Withdrawal times are calculated by performing a statistical tolerance limit procedure on residue data from the depletion curve of the drug residue in milk or a target tissue. The US FDA procedure predicts with 95% confidence a withdrawal time when the residue in milk or a target tissue in 99% of the animal population receiving the drug is at or below tolerance limits.9 Part of the approval process for a veterinary drug requires that residue studies be conducted in healthy animals. One assumption of the approval process is that there will be no change in the drug’s pharmacokinetics when administered to diseased animals, compared with results for healthy animals. However, disease states can profoundly alter the pharmacokinetic behavior of a drug.10-12 The most profound differences in pharmacokinetic responses are generally associated with hepatic, renal, and cardiovascular disease, but other processes such as inflammation, endotoxemia, and stress can also significantly alter a drug’s absorption, distribution, metabolism, and elimination.13 Although data are limited on the effects of disease on the pharmacokinetics of drugs in cattle, there is evidence to suggest that the use of healthy animals for establishment of drug withdrawal periods may not be appropriate. For example, a study14 in the late 1970s revealed that following intramammary infusion of several antimicrobials, only minimal concentrations were found in the kidneys and liver of healthy cows. Detectable concentrations of antimicrobials were found only in the liver and kidneys for 24 hours, and residues were not detected in the meat of these cattle. In contrast, antimicrobial concentrations in the tissues of mastitic cows were much higher and persisted longer. Differences in drug pharmacokinetics have been described for oxytetracycline in cows with theileriosis.15 Following IM administration, infected cattle had significantly prolonged absorption and terminal elimination half-life, MRT, AUC0-∞, and bioavailability, compared with results for healthy cows.16 Additionally, 5 of 20 calves with respiratory disease died after administration of theophylline during a field trial,17 whereas all 20 calves that received a placebo survived. A subsequent study18 revealed that calves with pneumonia had significantly higher plasma concentrations of theophylline, compared with concentrations in healthy calves. Similarly, greater secretion of ceftriaxone into milk was also detected in cows with endometritis after IV administration, compared with results for control cows.19 Finally, use of a population pharmacokinetic model revealed the need for a longer withdrawal interval than the FDA-appro
d approved withdrawal time20 for flunixin in meat, which suggested a need for pharmacokinetic studies in both healthy and diseased animals.

The present study provided strong evidence that withdrawal times for milk determined in healthy cattle may not be appropriate for cows with clinical mastitis. Pharmaceutical companies must conduct studies to determine the efficacy of various drugs for treating a specific disease or condition during the approval process, so it would appear logical that pharmacokinetic and residue studies could be performed with the same animals or under similar conditions.

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c. ToDAY, Boehringer Ingelheim Vetmedica, St Joseph, Mo.
e. Metatron sampler, Wessfalia Surge, Naperville, Ill.
f. Supelco hybrid SPE-phospholipid cartridge, Sigma-Aldrich, St Louis, Mo.
g. TurboVap LV evaporator, Zymark Corp, Hopkinton, Mass.
h. Acuity, Waters Corp, Milford, Mass.
i. Phoenix WinNonlin, version 1.3x, Pharsight Corp, St Louis, Mo.
j. SAS, version 9.1, SAS Institute Inc, Cary, NC.


