



Plasma pharmacokinetics and milk residues of flunixin and 5-hydroxy flunixin following different routes of administration in dairy cattle

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ABSTRACT

The objective of this study was to determine if the plasma pharmacokinetics and milk elimination of flunixin (FLU) and 5-hydroxy flunixin (5OH) differ following intramuscular and subcutaneous injection of FLU compared with intravenous injection. Twelve lactating Holstein cows were used in a randomized crossover design study. Cows were organized into 2 groups based on milk production (<20 or >30 kg of milk/d). All cattle were administered 2 doses of 1.1 mg of FLU/kg at 12-h intervals by intravenous, intramuscular, and subcutaneous injections. The washout period between routes of administration was 7 d. Blood samples were collected from the jugular vein before FLU administration and at various time points up to 36 h after the first dose of FLU. Composite milk samples were collected before FLU administration and twice daily for 5 d after the first dose of FLU. Samples were analyzed by ultra-HPLC with mass spectrometric detection. For FLU plasma samples, a difference in terminal half-life was observed among routes of administration. Harmonic mean terminal half-lives for FLU were 3.42, 4.48, and 5.39 h for intravenous, intramuscular, and subcutaneous injection, respectively. The mean bioavailability following intramuscular and subcutaneous dosing was 84.5 and 104.2%, respectively. The decrease in 5OH milk concentration versus time after last dose was analyzed with the nonlinear mixed effects modeling approach and indicated that both the route of administration and rate of milk production were significant covariates. The number of milk samples greater than the tolerance limit for each route of administration was also compared at each time point for statistical significance. Forty-eight hours after the first dose, 5OH milk concentrations were undetectable in all intravenously injected cows; however, one intramuscularly injected and one subcutaneously injected cow had measurable concentrations. These cows had 5OH concentrations above the

tolerance limit at the 36-h withdrawal time. The high number of FLU residues identified in cull dairy cows by the United States Department of Agriculture Food Safety Inspection Service is likely related to administration of the drug by an unapproved route. Cattle that received FLU by the approved (intravenous) route consistently eliminated the drug before the approved withdrawal times; however, residues can persist beyond these approved times following intramuscular or subcutaneous administration. Cows producing less than 20 kg of milk/d had altered FLU milk clearance, which may also contribute to violative FLU residues.

Key words: flunixin, residue, pharmacology

INTRODUCTION

Flunixin (FLU) is a nonsteroidal antiinflammatory drug (NSAID) licensed for use in beef and dairy cattle for modulation of inflammation in endotoxemia and for the control of pyrexia associated with bovine respiratory disease and acute bovine mastitis. Flunixin is labeled for intravenous administration at a dose of 2.2 mg/kg every 24 h or 1.1 mg/kg every 12 h. The slaughter withdrawal time is 4 d following the last injection and the milk withdrawal time is 36 h. Although FLU is only approved for intravenous administration; intramuscular and subcutaneous administrations are common routes of extra-label drug use in dairy cattle due to their ease of administration (FDA, 2004). However, altering the route of administration for convenience is not an appropriate reason for extra-label drug use according to the Animal Medicinal Drug Use Clarification Act (AMDUCA; FDA, 1996). Because intramuscular and subcutaneous administration of FLU are not approved in the United States, milk-withholding times have not been established. In addition, limited data is available that describes the pharmacokinetics of FLU after intramuscular or subcutaneous administration in cattle, which is necessary to provide guidance on withdrawal times following intramuscular and subcutaneous administration.

Altering the route of administration, formulation, and dose can affect the rate of elimination of a drug,

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resulting in violative residues (KuKanich et al., 2005; Gehring et al., 2006). The United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS) Red Book from 2005 through 2010 reported an increasing number of residue violations in meat from dairy cattle (USDA-FSIS, 2005–2010). In the last 5 yr, FLU has become the second most common residue violation behind penicillin in culled dairy cattle (USDA-FSIS, 2005–2010). Currently, milk is not tested for 5-hydroxy FLU (5OH), the marker residue for FLU in milk. However, in December 2011, the FDA announced plans to collect and test milk samples across the United States for 5OH residues. Because a significant number of FLU tissue residue violations are found in culled dairy cows, a concern exists that the same practices that lead to tissue residues might also be leading to drug residues in milk. This increase in violative FLU residues may be associated with administration of this drug by an extra-label route. The administration of FLU by intramuscular or subcutaneous routes may prolong drug elimination and result in milk concentrations of 5OH to be greater than the tolerance level of 2 µg/kg after 36 h. Flunixin is metabolized to 5OH in the liver and is excreted in both urine and feces; however, 0.0062 to 0.0124% of the administered dose is excreted as 5OH in milk (Lichtenwalner et al., 1986; FDA, 2004). Our hypothesis was that if FLU is administered via an extra-label route, then the plasma half-life and milk residues following intramuscular or subcutaneous injection may persist longer than the plasma half-life and milk residues following intravenous administration. Therefore, the primary objective of this study was to examine the plasma pharmacokinetics and milk residues of FLU and 5OH in lactating dairy cattle following administration by intravenous, intramuscular, and subcutaneous routes.

MATERIALS AND METHODS

This study was approved by the North Carolina State University (Raleigh) Institutional Animal Care and Use Committee.

Animals

Twelve lactating Holstein cows weighing between 545 and 676 kg were used in a randomized crossover design study. Cows were organized into 2 groups based on milk production. Group A consisted of 6 cows producing less than 20 kg of milk per day. Group B consisted of 6 cows producing greater than 30 kg of milk per day. Prior to the start of the trial, intravenous catheters were aseptically placed in the jugular vein.

Experimental Design

Flunixin was administered intravenously, intramuscularly, and subcutaneously in a randomized crossover design at a dose of 1.1 mg/kg, given in 2 doses at a 12-h interval. After 7-d washout periods, each group received FLU via each of the other routes (intravenous, intramuscular, or subcutaneous), so that by the study completion, each of the 12 cows had received FLU intravenously, intramuscularly, and subcutaneously. For the intravenous group, FLU was administered into the contralateral jugular vein to which the jugular catheter had been placed. All intramuscular and subcutaneous injections were given in the neck.

Blood and Milk Sampling

Blood samples were collected from the jugular catheter into heparinized tubes before FLU administration and at 0.25, 0.5, 1, 2, 4, 8, and 12 h after the first dose of FLU. Blood samples were also collected 12 and 24 h after the second dose of FLU. Blood samples were centrifuged at $1,690 \times g$ for 10 min at -15°C ; plasma was collected and frozen at -20°C until analysis of plasma FLU and metabolite concentrations. Prior to FLU administration, 5 mL of foremilk was manually collected from each quarter of every cow. Composite milk samples were collected using a Metatron sampler (Westfalia Surge Inc., Naperville, IL). Composite milk samples (50 mL) were collected at 1.5 and 12 h after the first dose of FLU and 12, 24, 36, 48, 72, 84, and 96 h after the second dose of FLU. Milk samples were immediately frozen at -20°C until analysis.

Sample Measurements

Flunixin and 5OH concentrations were quantified by ultra-HPLC with mass spectrometric detection. For plasma sample extraction, plasma samples were thawed, and 0.3 mL of plasma was combined with 0.9 mL of 0.5% citric acid in acetonitrile. Samples were sonicated for 5 min and then centrifuged for 10 min at $3,500 \times g$. The supernatant was loaded on a Supelco Hybrid SPE-phospholipid cartridge (Sigma-Aldrich, St. Louis, MO). The eluate from the cartridge was collected and placed in a 55°C TurboVap LV evaporator (Zymark Corp., Hopkinton, MA) to dryness under a 20-psi (137.9-kPa) 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 of the samples to those of an external standard curve made from spiked plasma samples put through the

sample cleanup process. For FLU and 5OH milk extraction, 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 process described previously for plasma was used for extraction and quantification.

The Acquity ultra performance liquid chromatography (UPLC)-MS system (Waters Corp., Milford, MA) consisted of an HSS T3 column (1.8- μm particle size, 2.1×100 mm) and filter disc. The mobile phase was acetonitrile: 0.1% acetic acid in water (68:32 vol/vol). The evaporative mass detector (EMD) 100 was a single quadrupole mass spectrometer run in positive electrospray ionization (ESI+) mode. Ions with mass-to-charge ratios of 297.0 and 313.0 were used for quantification of FLU and 5OH, respectively. The column temperature was 30°C and sample temperature was 4°C. Run times were 2.2 min. The limit of quantification (LOQ) was determined as 10 times the standard deviation of 6 blank samples. The limit of detection (LOD) was determined as 3 times the standard deviation of 6 blank samples. The LOD and LOQ for FLU and 5OH in plasma were 0.01 and 0.02 $\mu\text{g}/\text{mL}$, respectively, and the linear range was from 0.02 to 20 $\mu\text{g}/\text{mL}$. The LOD and LOQ for FLU and 5OH in milk were 0.001 and 0.002 $\mu\text{g}/\text{mL}$, respectively, and linear range for milk was 0.002 to 1 $\mu\text{g}/\text{mL}$. Relative standard deviations for both interday and intraday were <15% at all concentrations.

Analysis

A noncompartmental analysis of FLU and 5OH plasma concentration versus time profiles was performed with Phoenix pharmacokinetic modeling software (Pharsight Corp., St Louis, MO). For all routes of administration, the area under the plasma concentration-time curve from time zero to infinity ($\text{AUC}_{0-\infty}$) and the area under the first moment curve (AUMC) were calculated by the linear trapezoidal rule for the first dose only. The rate constant (λ_z), associated with the terminal elimination phase, was estimated by means of linear regression of the terminal phase of the log concentration versus time profile, and the corresponding terminal half-life ($t_{1/2\lambda_z}$) for intravenous, intramuscular, and subcutaneous routes of administration were calculated. The rate constant also was used to extrapolate $\text{AUC}_{0-\infty}$ and AUMC from the time of the last observed concentration to infinity for all routes of administration. For intravenous administration, the volume of distribution at steady state (Vd_{ss}) and volume of distribution for the terminal elimination phase (Vd_{area}) were calculated. The $\text{AUC}_{0-\infty}$ and AUMC were used to calculate clearance (CL), and mean residence time for intravenous administration. For extravascular routes of administration, $\text{AUC}_{0-\infty}$ and AUMC were used to

calculate mean transit time (MTT), mean absorption time (MAT), and bioavailability (F).

Statistical Analysis

All values are expressed as mean \pm standard deviation, with the exception of $t_{1/2\lambda_z}$, which is expressed as the harmonic mean \pm standard deviation. Pharmacokinetic parameters for FLU and 5OH in plasma, and 5OH milk concentrations were compared by a one-way ANOVA with the Tukey test, where $P < 0.05$ was considered statistically significant. Mean transit times, MAT, and F were compared using a Student's t -test, where $P < 0.05$ was considered statistically significant. For comparison of the number of 5OH milk samples greater than the 2- $\mu\text{g}/\text{kg}$ tolerance limit following intravenous, intramuscular, and subcutaneous administration, the differences in frequency of detectable concentrations at each time point among the routes of administration were evaluated with the Cochran Q test. For the test, the milk concentrations of 5OH at each time point were coded as 0 for undetectable and 1 for greater than the tolerance limit. The statistical analyses were performed with SAS software (SAS Institute Inc., Cary, NC). The decrease in 5OH milk concentration versus time after last dose was analyzed with the nonlinear mixed effects modeling approach as implemented with Phoenix (Pharsight Corp.) to determine if variability in the rate was significantly affected by either route of administration or rate of milk production.

RESULTS AND DISCUSSION

Plasma

The objective of this study was to determine if the plasma pharmacokinetics and milk elimination of FLU and 5OH in lactating dairy cattle would differ following intramuscular and subcutaneous injection of FLU as compared with intravenous administration. Table 1 presents the plasma pharmacokinetic parameters after intravenous, intramuscular, and subcutaneous dosing. Following intravenous administration, mean FLU plasma concentrations decreased from 8.8 to ≤ 0.1 $\mu\text{g}/\text{mL}$ by 12 h after the first dose (Figure 1). Following intramuscular administration, the mean observed peak plasma concentration (C_{max}) was 2.2 ± 0.96 $\mu\text{g}/\text{mL}$, and the observed time to maximum concentration (T_{max}) occurred 0.25 to 0.5 h after injection. Following subcutaneous administration, C_{max} was 1.33 ± 0.65 $\mu\text{g}/\text{mL}$ and T_{max} was observed 0.25 to 2 h after dosing. Twenty-four hours after the second dose, 50% of the cows that received FLU intramuscularly or subcutaneously had detectable FLU plasma concentrations;

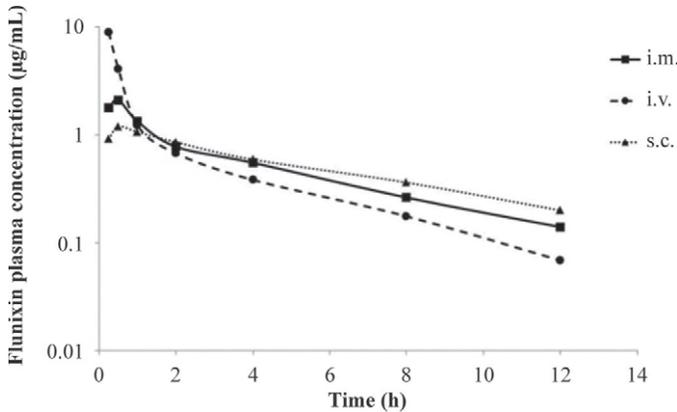


Figure 1. Mean plasma concentration of flunixin following the first dose of flunixin by intravenous, intramuscular, and subcutaneous administration.

however, FLU was not detected in the plasma of any of the cows 24 h after intravenous administration (data not shown).

A difference in the terminal $t_{1/2\lambda z}$ for FLU was observed among routes of administration. The $t_{1/2\lambda z}$ of FLU in plasma following subcutaneous administration was significantly longer ($P < 0.05$) compared with the $t_{1/2\lambda z}$ following intravenous administration. The prolonged $t_{1/2\lambda z}$ observed following subcutaneous administration may be due to delayed absorption affecting the terminal phase. Because the last time point sampled was 12 h after injection, a greater likelihood exists of seeing an effect on absorption. The $t_{1/2\lambda z}$ in the current study was also shorter than the $t_{1/2\lambda z}$ (7.46 h) reported

by Lacroix et al. (2011) following subcutaneous administration of FLU. This difference may be attributed to a shorter sample-collection period in the current study (12 h after dosing) compared with the 60 h post-dosing sample time of the Lacroix et al. (2011) study. The MTT and MAT did not differ significantly ($P = 0.08$ and $P = 0.09$) across routes of administration in the current study; however, significant differences in absorption between intramuscular and subcutaneous routes has been reported in the literature (FDA, 2010). One limitation of the current study was that a large percentage (up to 32%) of $AUC_{0 \rightarrow \infty}$ was extrapolated for several of the cows receiving FLU by intramuscular and subcutaneous administration. Because $AUC_{0 \rightarrow \infty}$ was used to calculate MTT, MAT, and F, a greater degree of variability exists in those parameters. This limitation was unavoidable because giving the entire dose of FLU (2.2 mg/kg) in a single injection may have resulted in tissue necrosis from intramuscular administration (Pyörälä et al., 1999; Smith et al., 2008); therefore, the dose was divided into 2 doses of 1.1 mg/kg and samples could only be collected up to 12 h after the first dose before administration of the second dose. In the present study, the mean plasma $t_{1/2\lambda z}$ for FLU after intravenous dosing was 3.42 h, which corresponds with that found in previous studies (Benitz, 1984; Anderson et al., 1990; Odensvik and Johansson, 1995; Jaroszewski et al., 2008; Abo-El-Sooud and Al-Anati, 2011; Wu et al., 2012). However, other studies have reported $t_{1/2\lambda z}$ (5.2–8.12 h) much longer than the 3.42 h $t_{1/2\lambda z}$ in the current study (Hardee et al., 1985; Landoni et al., 1995; Odensvik, 1995; Rantala et al., 2002). This variability may be

Table 1. Flunixin plasma pharmacokinetic parameters after intravenous, intramuscular, and subcutaneous dosing

Parameter ¹	Route of administration		
	i.v.	i.m.	s.c.
$t_{1/2\lambda z}$ (h)	3.42 ± 0.98 ^{a*}	4.48 ± 1.77 ^{ab*}	5.39 ± 2.47 ^{b*}
λ (h ⁻¹)	0.219 ± 0.065 ^{a*}	0.175 ± 0.06 ^{ab*}	0.151 ± 0.061 ^{b*}
$AUC_{0 \rightarrow \infty}$ (µg·h/mL)	7.99 ± 2.72	7.36 ± 3.3	8.29 ± 4.15
$AUC_{0 \rightarrow t_{last}}$ (µg·h/mL)	7.63 ± 2.66	6.37 ± 2.93	6.36 ± 2.34
CL (mL/h per kilogram)	150.6 ± 43.1		
Vd_{ss} (L/kg)	0.254 ± 0.143		
Vd_{area} (L/kg)	0.757 ± 0.361		
MTT ² (h)	2.80 ± 0.80	5.51 ± 2.27	7.58 ± 3.44
MAT* (h)		2.71 ± 2.24	4.78 ± 3.17
F (%)		84.5 ± 28.9	104.2 ± 37.2

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹ $t_{1/2\lambda z}$ = harmonic mean elimination half-life; λ = rate constant associated with the terminal elimination phase; $AUC_{0 \rightarrow \infty}$ = total area under the curve; $AUC_{0 \rightarrow t_{last}}$ = area under the curve from 0 to the last observed concentration; CL = clearance; Vd_{ss} = volume of distribution at steady state; Vd_{area} = volume of distribution in terminal elimination phase; MTT = mean transit time; MAT = mean absorption time; F = bioavailability. All results are expressed as mean ± SD, except $t_{1/2\lambda z}$, which is expressed as harmonic mean ± SD.

²Mean residence time for i.v. administration.

* $P < 0.05$.

explained by the time points used for determination of $t_{1/2\lambda_z}$. In a study by Landoni et al. (1995), time points up to 36 h were used to calculate $t_{1/2\lambda_z}$ resulting in a $t_{1/2\lambda_z}$ of 6.87 h. In the current study, mean $AUC_{0\rightarrow\infty}$ following intravenous dosing at 1.1 mg/kg was half the $AUC_{0\rightarrow\infty}$ values reported in the literature, in which 2.2 mg/kg was administered intravenously (Anderson et al., 1990; Odensvik and Johansson, 1995). The CL calculated in the current study is similar with findings in the literature (Hardee et al., 1985; Anderson et al., 1990; Landoni et al., 1995; Odensvik, 1995; Odensvik and Johansson, 1995; Rantala et al., 2002). The $V_{d_{area}}$ and $V_{d_{ss}}$ in the current study were greater than expected for a drug that is highly (99%) protein bound; however, the values were consistent with the literature (Hardee et al., 1985; Anderson et al., 1990; Landoni et al., 1995; Odensvik, 1995; Odensvik and Johansson, 1995; FDA, 1998; Rantala et al., 2002). Both $V_{d_{area}}$ and $V_{d_{ss}}$ were at the lower end of the range of values reported in the literature because of the large percentage of $AUC_{0\rightarrow\infty}$ that was extrapolated in the current study. Multiple studies have described FLU with a multi-compartment pharmacokinetic model and have reported peripheral distribution despite high protein binding of FLU (Anderson et al., 1990; Odensvik and Johansson, 1995; Buur et al., 2006).

The mean F following intramuscular and subcutaneous dosing was 84.5 and 104.2%, respectively. Variability in F was noted in this study and may again be due to the large percentage of $AUC_{0\rightarrow\infty}$ that was extrapolated. However, the F reported in the current study for intramuscular administration corresponds to the F (76%) reported by Anderson et al. (1990) in cattle and F (79%) reported by Königsson et al. (2003) in goats.

Table 2 presents 5OH plasma $t_{1/2\lambda_z}$ following intravenous, intramuscular, and subcutaneous dosing. Regardless of route of administration, plasma 5OH concentrations were greatest between 0.25 to 0.5 h after FLU administration. A significant difference between plasma FLU and 5OH $t_{1/2\lambda_z}$ was noted, with 5OH $t_{1/2\lambda_z}$ being shorter than FLU $t_{1/2\lambda_z}$ following intravenous administration of FLU. The shorter $t_{1/2\lambda_z}$ for 5OH, compared with the $t_{1/2\lambda_z}$ for FLU, was most likely due to an inadequate concentration-versus-time profile for 5OH in plasma caused by samples being below the LOD within 4 h after the first dose and, thus, the $t_{1/2\lambda_z}$ reflected the distribution phase rather than the terminal elimination phase (Bonate and Howard, 2004).

Milk

The nonlinear mixed effects modeling of the decrease in 5OH milk concentrations over time indicated that

both the route of administration ($P < 0.001$) and rate of milk production ($P < 0.1$) were significant covariates. In this study, cows producing less than 20 kg of milk/d eliminated 5OH slower than cows producing greater than 30 kg of milk/d. Research describing the effect of milk production on drug elimination is limited, especially for systemically administered drugs. However, several studies have shown a correlation between low milk production and prolonged drug elimination for some intramammary drugs (Mercer et al., 1970; Whittem, 1999; Smith et al., 2004; Gehring and Smith, 2006; Stockler et al., 2009). One study with 21 different commercially available intramammary products found that cows producing less than 9 kg of milk/d were more likely to have prolonged withholding times than higher-producing cows (Mercer et al., 1970). However, level of milk production may only partially explain variations in excretion rates as several high-producing cows were reported to have slow drug elimination (Mercer et al., 1970; Lainesse et al., 2012). Table 3 presents 5OH milk concentrations and the number of cows with milk concentrations greater than the tolerance limit of 2 $\mu\text{g}/\text{kg}$ following intravenous, intramuscular, and subcutaneous administration at various sampling times. At each time point, 5OH milk concentrations were compared by route of administration. Mean 5OH milk concentrations 1.5 h after injection for intravenous, intramuscular, and subcutaneous injections were 0.044, 0.029, and 0.02 $\mu\text{g}/\text{mL}$, respectively, indicating that FLU was rapidly metabolized to 5OH and entered the milk shortly after FLU administration. Following intramuscular and subcutaneous FLU administration, mean 5OH milk concentrations at 1.5 h were significantly less than 5OH milk concentrations following intravenous FLU administration. The difference in mean 5OH milk concentrations between intravenous and extravascular routes can be attributed to the lower plasma concentrations, which resulted from differences in absorption of FLU. Differences ($P < 0.05$) in 5OH milk concentrations were also detected 12 h after the first dose, but not 12 h after the second dose. The number of milk samples where 5OH could be detected above a con-

Table 2. 5-Hydroxy flunixin plasma elimination half-life ($t_{1/2\lambda_z}$; harmonic mean \pm SD) after intravenous, intramuscular, and subcutaneous dosing

Parameter	Route of administration		
	i.v.	i.m.	s.c.
$t_{1/2\lambda_z}$ (h)	1.44 \pm 0.657 ^a	6.0 \pm 6.61 ^b	7.01 \pm 2.22 ^b

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

Table 3. Milk concentrations ($\mu\text{g/mL}$) of 5-hydroxy flunixin (mean \pm SD) and number of positive samples (samples greater than 2 $\mu\text{g/kg}$) following intravenous, intramuscular, and subcutaneous administration of flunixin

Time (h)	Concentration ¹			Number of positive samples ²		
	i.v.	i.m.	s.c.	i.v.	i.m.	s.c.
1.5	0.044 \pm 0.02 ^A	0.029 \pm 0.021 ^B	0.021 \pm 0.011 ^B	12 ^a	11 ^b	12 ^a
12	0.010 \pm 0.004 ^A	0.020 \pm 0.013 ^B	0.022 \pm 0.017 ^B	12	12	12
24	0.009 \pm 0.005	0.018 \pm 0.008	0.016 \pm 0.010	10 ^a	11 ^b	12 ^b
36	0.005	0.004 \pm 0.001	0.004 \pm 0.001	1 ^a	8 ^b	8 ^b
48		0.002	0.0022		1	1

^{A,B}Means within a row with different superscripts differ ($P < 0.05$).

^{a,b}Number of positive samples within a row with different superscripts differ ($P < 0.001$).

¹Compared by ANOVA with the post hoc Tukey test.

²Compared with the Cochran Q test.

centration of 2 $\mu\text{g/kg}$ for each route of administration was also compared at each time point for statistical significance. The number of milk samples where 5OH could be detected above the tolerance limit 1.5 h after the first dose differed across routes of administration, with all cows having greater than 2 $\mu\text{g/kg}$ of 5OH in their milk. One exception occurred, which was a cow that received FLU by intramuscular administration. Forty-eight hours after the first dose (36 h after the second injection), 5OH milk concentrations were undetectable in all intravenously injected cows; however, 1 intramuscularly and 1 subcutaneously injected cow had concentrations greater than the tolerance limit. These cows still had milk 5OH concentrations above 2 $\mu\text{g/kg}$ at the 36-h withdrawal time, indicating that administration of FLU via an extra-label route may affect milk CL of FLU and potentially result in violative milk residues.

The presence of drug residues in milk is a primary concern for the dairy industry. A survey of food animal veterinarians conducted in 1992, found that 93% of the veterinarians used NSAID and of that 93%, almost 60% reported using these drugs more than once per week (Kopcha et al., 1992). In 1995, a survey of dairy veterinarians reported antiinflammatory drugs to be the second-most-prescribed class of drugs after antimicrobials (Sundlof et al., 1995). Also, a recently published study reported NSAID to be one of the most frequently administered analgesics in cattle in the United States (Fajt et al., 2011). Milk residues from NSAID and antimicrobials result in significant economic losses to the producer and pose a potential health hazard to the consumer. Strict financial penalties and suspension of the producer's grade "A" permit are possible outcomes of drug residues detected in milk. To prevent economic losses to the producer, it is imperative to administer FLU by the labeled route (intravenous) and observe the proper milk withholding time of 36 h.

CONCLUSIONS

The high number of FLU residues identified in culled dairy cows by the USDA-FSIS is likely related to administration of the drug by an unapproved route. Cattle that received FLU by the approved route (intravenous) consistently eliminated the drug before the approved withdrawal times; however, residues can persist beyond these approved times following intramuscular or subcutaneous drug administration. Education of veterinarians and farm personnel in proper drug administration is critical in the prevention of milk residue violations.

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