Plasma, Urine and Tissue Concentrations of Flunixin and Meloxicam in Pigs

Emma Nixon
North Carolina State University
Travis P. Mays
Texas A&M University College Station
Patricia A. Routh
North Carolina State University
James L. Yeatts
North Carolina State University
Virginia R. Fajt
Texas A&M University College Station
Thomas Hairgrove
Texas A&M University College Station
Ronald Baynes (Ronald_Baynes@ncsu.edu)
North Carolina State University https://orcid.org/0000-0002-7920-9338

Research article

Keywords: Pig, Flunixin, Meloxicam, Pharmacokinetics, NSAIDs, Residue, Urine, Show

Posted Date: March 24th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-18726/v1

Version of Record: A version of this preprint was published on September 16th, 2020. See the published version at https://doi.org/10.1186/s12917-020-02556-4.

Abstract

Background: The objective of this study was to compare plasma and urine concentrations of flunixin and meloxicam in pigs, in order to determine urine withdrawal intervals for animals at livestock shows where urine is routinely tested for these drugs. Fourteen Yorkshire/Landrace cross pigs were housed in individual metabolism cages to facilitate complete urine collection. All animals were randomly divided into one of two treatment groups and received either 2.2 mg/kg flunixin or 0.4 mg/kg meloxicam via intramuscular injection. Plasma and urine were collected and analyzed by UPLC-MS/MS. Pigs were humanely euthanized when meloxicam/flunixin could no longer be detected in two consecutive urine samples, and liver and kidneys were collected to quantify any potential residues.

Results: Drug levels in urine reached peak concentrations between 4 and 8 h post-dose for both flunixin and meloxicam. Flunixin urine concentrations were higher than maximum levels determined in plasma. Urine concentrations for flunixin and meloxicam were last detected above the limit of quantification (LOQ) at 120 h (0.0005 µg/mL) and 48 h (0.001 µg/mL), respectively. Mean apparent elimination half-life in plasma was 5.00 ± 1.89 h and 3.22 ± 1.52 h for flunixin and meloxicam, respectively. Six of seven pigs had detectable liver concentrations of flunixin (range 0.0001-0.0012 µg/g) following negative urine samples at 96 and 168 h, however all samples at 168 h were below the FDA tolerance level (0.03 µg/g). Meloxicam was detected in a single liver sample at necropsy (0.0054 µg/g) at 72 h but was below the EU MRL (0.065 µg/g) which was used to determine a safety level when FDA tolerance or FSIS testing limits were not established. Urine concentrations did not relate to liver residues at the single slaughter time

Conclusions: This data suggests that pigs given a single intramuscular dose of meloxicam at 0.4 mg/kg or flunixin at 2.2 mg/kg are unlikely to have tissue residues above the US FDA tolerance or EU MRL following negative urine testing. This information will assist in generating withdrawal intervals for these NSAIDs for show pigs that might undergo a urine test by the livestock show authorities.

1 Background

Withdrawal intervals for veterinary drugs in livestock animals for the purposes of protecting human health from excessive exposure to those drugs are based on tissue concentrations. A withdrawal interval is the time required after administration of a drug to a food-producing animal for tissue concentrations of a drug or metabolite to fall below the tolerance level established by the responsible government authority, such as the FDA in the US or the EMA in Europe. The tolerance or minimum residue level is a specific concentration that is considered safe for human consumption (1). This is a useful approach for post-mortem determination of the safety of edible muscle tissue, but the challenge is to provide an approach for predicting tissue concentrations in live animals. Owners or veterinarians are not able to make quick, informed decisions regarding animals used in shows or exhibitions, where urine is often tested for the presence of measurable drug concentrations. Livestock show authorities drug test show animals in order to protect the safety of the food supply and to ensure fair competition. One of the groups of drugs of importance in this setting is nonsteroidal anti-inflammatory drugs (NSAIDs), which may be used to conceal an injury that would otherwise prevent a show animal from competing. However, urine concentrations of drugs correlate in an unknown manner with plasma or tissue concentrations, particularly for drugs for which renal clearance is only a minor portion of the total

clearance. Therefore, using urine concentrations to assess potential tissue residues could lead to inaccurate predictions of target tissue concentrations (2,3).

There is only one FDA-approved NSAID for use in pigs: flunixin meglumine for the control of pyrexia associated with swine respiratory disease. Another commonly prescribed NSAID, meloxicam, is not approved for use in the US in pigs, but is in the EU and Canada. The FDA has an established tolerance for flunixin in pigs, which is 30 ppb in liver (the target tissue) and 25 ppb in muscle (5). No tolerance has been established for meloxicam in pigs, and therefore detection of any tissue residue would be considered a violation by USDA Food Safety and Inspection Service (FSIS). The FDA tolerance is based on edible tissues due to food safety concerns, and there are no established tolerance limits in urine as urine is not of a food safety concern.

Limited data are available regarding the relationship between urinary excretion of a drug and the plasma or tissue concentrations. Urine concentrations of flunixin have previously been examined in cattle, goats, horses, camels and dogs (2,3,6–9), as well as pigs (10,11). Of the two studies that examined flunixin in urine of pigs, only one of those attempted to establish a correlation with tissue concentrations. However, that study used a single spot urine sample taken at necropsy to predict the residue depletion profile in edible tissues. Those results showed that earlier urine samples (24 h) were highly variable in concentration and are affected by many factors, including the urinary output, voiding intervals, last voiding time, postvoid residual urine volume (10,12). The author recommended that future studies should consider using metabolism cages to collect cumulative urine samples to improve the prediction of tissue concentrations. The second study (11) calculated 6.8% of the parent drug was excreted in urine following administration of 2.2 mg/kg flunixin intramuscularly once daily for 3 days in 3 month old pigs. However this study did not assess tissue concentrations of flunixin in pigs. There are far fewer data available regarding meloxicam concentration-time profiles in urine in large animal species, with only two studies in horses and goats (2,13), and no published studies have assessed the correlation between meloxicam concentrations in plasma, tissues and urine in pigs.

The objective of this study was to compare plasma and tissue (interstitial fluid; ISF) concentrations to urine concentrations of both meloxicam and flunixin, and to determine whether these NSAIDs are detectable in the liver or kidneys once the drugs are undetectable in the urine. Generally, in other veterinary species, NSAIDs demonstrate linear pharmacokinetics, therefore it was expected that urine and plasma concentration profiles would be comparable.

2 Results

All pigs (n = 7 for each treatment) completed the study with no adverse effects. One pig from the pilot study was excluded from the flunixin urine results due to carryover in the collection tray. One pig was excluded from the flunixin plasma and ISF results due to loss of the catheter and ISF probe.

2.1 Flunixin

2.1.1 Plasma and ISF

Mean plasma and ISF flunixin concentrations over time following a single IM injection of 2.2 mg/kg are presented in Fig. 1. Parameters describing the pharmacokinetics of flunixin following a single IM injection are presented in Table 1. Flunixin concentrations in plasma were last detected above the LOQ of 0.0005 µg/mL at 60 h. The plasma pharmacokinetics of flunixin after IM administration were characterized by rapid absorption, large volume of distribution/F (3.13 ± 1.82 L/kg) and an apparent elimination half-life (5.00 ± 1.89 h) which was relatively short. In interstitial fluid, the average maximum concentration of flunixin (Cmax) was 0.0039 µg/mL at 6 hours (Tmax) after flunixin administration.

Table 1

Plasma pharmacokinetic parameters following intramuscular administration of either flunixin meglumine 2.2 mg/kg (n = 6) or meloxicam 0.4 mg/kg (n = 7). Data are shown as mean ± standard deviation (for MRT, T1/2 and z, harmonic mean was calculated). MRT; mean residence time, T1/2; apparent elimination half-life, z; slope of the terminal phase, Tmax; time to maximal concentration, Cmax; maximum concentration, AUC; area under the concentration-time curve, Vd/F; volume of distribution per fraction absorbed, Cl/F; total body clearance per fraction absorbed.

Plasma Pharmacokinetic Parameters					
Parameter	Units	Flunixin		Meloxicam	
		Mean	±SD	Mean	±SD
Dose	mg/kg	2.20		0.40	
MRT	h	5.38	(1.69)	4.00	(2.23)
$T_{1/2}$	h	5.00	(1.89)	3.22	(1.52)
λ_z	h	0.12	(0.05)	0.20	(0.06)
T_{max}	h	0.33	(0.32)	0.51	(0.68)
C_{max}	µg/mL	2.20	(0.40)	1.34	(0.76)
AUC_{last}	h*µg/mL	6.26	(1.44)	5.02	(1.91)
AUC_{inf}	h*µg/mL	6.27	(1.44)	5.05	(1.94)
AUC_{extrap}	%	0.24	(0.08)	0.44	(0.43)
Vd/F	L/kg	3.13	(1.82)	0.42	(0.11)
Cl/F	L/h/kg	0.37	(0.10)	0.09	(0.03)

2.1.2 Urine

The highest concentration of flunixin in urine (Cmax; 1.55 ± 0.96 µg/mL) was detected in the first sample collected from each pig (Tmax; 5.33 ± 3.27 h, as not all pigs urinated for the first 4 h sample). After 120 h, urine concentrations for all pigs fell below the LOQ of 0.0005 µg/mL. Renal clearance for flunixin was 5.29 ± 2.98 mL/h/kg (Table 2).

Table 2

Renal clearance values, % contribution of renal clearance to total systemic clearance (Cl/F) and % total dose excreted in urine following intramuscular administration of 2.2 mg/kg flunixin (n = 5) or 0.4 mg/kg meloxicam (n = 7).

Renal Clearance					
Parameter	Units	Flunixin		Meloxicam	
Renal Clearance	mL/h/kg	5.29	(2.98)	0.17	(0.04)
Renal clearance component of Cl/F	%	1.39	(0.42)	0.20	(0.06)
Percent total dose excreted in urine as parent drug	%	1.39	(0.42)	0.20	(0.06)

2.1.3 Liver and Kidney

Flunixin was detected in liver samples from six out of seven pigs at necropsy following two consecutive negative urine samples (96–168 h; concentration range 0.0001–0.0012 µg/g). However, flunixin concentrations in all liver samples were far below the FDA tolerance of 0.03 µg/g (5) and EMA MRL of 0.2 µg/g (14). Only one kidney sample tested positive for flunixin (right kidney 0.0002 µg/g). Although there is no FDA tolerance level for kidney, this was far below the FSIS confirmatory limit of detection of 0.0125 µg/g (15) and EMA MRL of 0.03 µg/g (14).

2.2 Meloxicam

2.2.1 Plasma and ISF

Mean plasma and ISF meloxicam concentrations over time following a single IM injection of 0.4 mg/kg are presented in Fig. 2. Parameters describing the pharmacokinetics of meloxicam following a single IM injection are presented in Table 1. Meloxicam concentrations in plasma were last detected above the LOQ of 0.001 µg/mL at 36 h. The plasma pharmacokinetics of meloxicam after IM administration were characterized by rapid absorption and a relatively short apparent elimination half-life (3.22 ± 1.52 h). In interstitial fluid, the average maximum concentration of meloxicam (Cmax) was 0.0078 µg/mL at 10.5 hours (Tmax) after meloxicam administration.

2.2.2 Urine

The highest concentration of meloxicam in urine (Cmax; 0.05 ± 0.01 µg/mL) was detected in the first sample collected from each pig (Tmax; 4.57 ± 1.51 h, as not all pigs urinated for the first 4 h sample). After 48 h, urine concentrations for all pigs fell below the LOQ of 0.001 µg/mL. Renal clearance for meloxicam was 0.17 ± 0.04 mL/h/kg (Table 2).

2.2.3 Liver and Kidney

Meloxicam was detected in a single liver sample at necropsy following two consecutive negative urine samples (36–72 h; caudal lobe 0.0054 µg/g, although meloxicam was not detected in samples taken from other lobes of this pig's liver). While there is no FDA tolerance for meloxicam in pigs, this was below the EMA MRL of 0.065 µg/g (16). Meloxicam was not detected in any kidney sample.

3 Discussion

3.1 Plasma pharmacokinetics

Following intramuscular administration of 2.2 mg/kg flunixin, the peak plasma concentration was reached in approximately 20 minutes (0.33 h), which was slightly less than the previously reported Tmax of 0.61 h and 0.85 h following intramuscular administration to gilts (17) and 6-day-old piglets (18), respectively. The apparent elimination half-life (5.00 h) was similar to previously reported $t_{1/2}$ in 10-day-old piglets following intravenous administration of 2.2 mg/kg and 4.4 mg/kg flunixin (4.82 h and 5.15 h, respectively (19)), but less than previously reported in gilts and 6-day-old piglets administered 2.2 mg/kg intramuscularly (7.49 h and 7.93 h (17,18). Elimination half-life is a dependent variable and can be altered by changes in drug distribution and clearance. Variation between studies (including multiple injection sites in the gilt study) could be responsible for these differences in half-lives.

Only one previous study reported the volume of distribution/F following an intramuscular dose of flunixin, and the reported value was much less than the Vd/F in the present study (0.92 L/kg for 6-day-old piglets (18) compared to 3.13 L/kg in this study). The Vd/F is affected by the degree of plasma and tissue protein binding, as well as the drug's lipophilicity (20). Lipid-soluble drugs such as flunixin have high apparent volumes of distribution, and flunixin has been shown to be highly bound to plasma proteins in pigs (99% (21)). The body fat-to-water ratio increases with age, resulting in increased sequestration of flunixin in adipose tissue (20), which may explain the variation in Vd/F between 6-day-old piglets and the juvenile pigs in the present study, both administered 2.2 mg/kg flunixin intramuscularly. The current Vd/F is also greater than previously reported Vd following IV doses in 10-day-old piglets (0.25 L/kg and 0.26 L/kg, (19)), juvenile pigs (18–27 kg body weight) following IV dose (1.83 L/kg, (21)) and mature sows (0.91 L/kg; (17)). Although the reason for these differences is unknown, variation maybe attributed to age, genetic differences, body condition, or volume, number, or location of injection sites.

Following intramuscular administration of 0.4 mg/kg meloxicam, the peak plasma concentration was reached around 30 minutes (0.51 h), which was similar to previously reported in 8-day-old piglets given a dose of 1 mg/kg (0.50 h (22)), but much quicker than reported in 5-day-old piglets given 0.4 mg/kg and 2-week-old piglets given 0.6 mg/kg (1.21 h and 1.1 h, respectively (18,23). However, the apparent elimination half-life was comparable to most previous reports (3.22 h in this study compared to 2.6 h, 3.94 h and 4.46 h; (18,22,23)), except for the elimination half-life reported in sows following an intravenous dose of 0.5 mg/kg (6.15 h; (24)). Despite differences in routes of administration, this difference may suggest that drug elimination may be slower in mature pigs.

Volume of distribution/F in this study for meloxicam (0.42 L/kg) was comparable to that of other studies investigating piglets 5–23 days of age, as well as mature sows, given doses in the range of 0.4-1.0 mg/kg and given via intramuscular or intravenous routes of administration (18,19,22,24,25).

3.2 Urine pharmacokinetics and renal clearance

As a general rule, NSAIDs are primarily eliminated by hepatic biotransformation, with renal excretion of the parent compound contributing to a small amount of total excretion (< 5% (26)). In this study, the percent of the total dose that was excreted as unchanged parent drug in the urine was low for both flunixin and meloxicam (1.39% and 0.20%, respectively). The total body clearance for each of these NSAIDs was comparable to previous studies (19,21–23,25), and represents elimination from the whole body, including hepatic and renal elimination. The relative contribution of renal clearance to the overall systemic clearance was low, suggesting that the main route of elimination is hepatic metabolism, although this study is limited in that the metabolites of either NSAID were not measured. Renal clearance of flunixin or meloxicam has not been measured in pigs previously.

The relationship between plasma and urine flunixin concentrations across all time points indicates that urine flunixin concentrations were higher than those measured in plasma at any given time point, similar to previously reported in both cattle and goats (2,6), however this was the opposite for meloxicam, with plasma concentrations being similar to or higher than that of the urine, again, similar to previously reported in goats (2). Glomerular filtration rate of 7-week-old pigs has been reported at 4.31 mL/min/kg (258.6 mL/h/kg; (27)), which is greater than the renal clearance values for both meloxicam and flunixin in this study, indicating that clearance of these NSAIDs is mainly via glomerular filtration with tubular reabsorption.

3.3 Tissue residues

The FDA established tolerance levels are based on edible tissues, or the slowest depleting organs (target tissues) which often refers to the liver or kidneys. For livestock shows, it is not possible to directly test these target tissues, and plasma samples are not convenient, and urine is tested instead. Detection of drug in urine may be a violation of livestock show rules.

Interstitial fluid (ISF) was collected to create a concentration-time profile in an attempt to reflect the tissue concentrations across multiple time points for each pig. However, these concentrations did not correlate well with plasma or urine concentrations, particularly for flunixin in which the ISF concentrations were prolonged but at a low level. However, the ISF concentrations for both meloxicam and flunixin fell below the LOQ before the urine concentrations.

Flunixin concentrations detected in the liver following negative urine samples were far below the FDA tolerance and present no food safety concerns or violations of livestock show rules. Meloxicam was detected in a single liver sample at necropsy. While there is no FDA tolerance for meloxicam in pigs, this was below the EMA MRL for meloxicam in liver. However, the presence of meloxicam at any level in pig liver in the US would be a violation according to US FSIS.

While the liver is regarded as the main target tissue when examining residues of NSAIDs, this study also measured drug concentrations in the kidneys. Only one kidney sample tested positive for flunixin. Although

there is no FDA tolerance level for kidney, the concentration detected was far below the FSIS confirmatory limit of detection. However, limits of detection change as analytical methods improve. Any detectable amount of flunixin in the kidneys would technically be violative. Meloxicam was not detected in any kidney sample.

4 Conclusions

NSAIDs are used in an extralabel manner for treating painful or inflammatory conditions in pigs, including but not limited to lameness, and could be used to mask an injury in a competing show animal. In this study, liver and kidney concentrations fell below either the FDA tolerance or the detection level of current FSIS testing methods after negative urine samples. However, this study was not able to determine the converse, i.e., whether detectable concentrations of meloxicam or flunixin in the urine correlated with drug concentrations above the tolerance or above detectable levels in the liver. While flunixin does hold a label for use in pigs, currently, any detectable level of flunixin found in the urine of pigs at livestock shows can disqualify the exhibitor. Label withdrawal times are based on the amount of time that must pass for edible tissues to be safe for human consumption, which may be less than the amount of time taken for all detectable drug to be eliminated. According to the present study, following a single intramuscular dose of 2.2 mg/kg flunixin, the drug should be undetectable in the urine if the label withdrawal time (12 days) is followed appropriately. There is no approved label in the US and therefore no established withdrawal time for meloxicam in pigs; however, this study suggests that pigs given a single intramuscular dose 0.4 mg/kg may test positive in urine for up to 2 days post-dose. On the other hand, following negative urine samples, meloxicam may still be detected in the liver (albeit below current FSIS testing limits). As there is no label for meloxicam in pigs, any level detected in the tissues would be considered violative in the US. This study provides useful information that can help livestock show authorities and veterinarians determine an appropriate elimination period for show animals whose urine may be tested prior to competition, and it may help provide data on which to base penalties for detection of flunixin or meloxicam in urine in show pigs.

5 Methods

5.1 Animals and housing

North Carolina State University Institutional Animal Care and Use Committee approved this study. A total of fourteen healthy, castrated, male Yorkshire/Landrace cross pigs (weighing 23.1–35.4 kg) were enrolled and randomly assigned to receive either flunixin or meloxicam (n = 7 per treatment group). These are typical number of animals used in a similar pharmacokinetic study in food animals. All animals were acquired from the North Carolina State University Swine Education Unit and transferred to the North Carolina State University College of Veterinary Medicine, where they were housed individually in metabolism cages (72^{o}F), with a 12:12 light:dark cycle, fed LabDiet 5084 (LabDiet, St. Louis, MO, USA) twice a day and had access to freshwater ad libitum.

5.2 Catheter and Interstitial Probe Placement

Prior to the start of the study, pigs were moved to individual metabolism cages and allowed 4 days of acclimation. After the adjustment period pigs were sedated using an intramuscular injection of a combination

of Telazol® (50 mg/mL tiletamine HCl and 50 mg/mL zolazepam HCl), ketamine (100 mg/mL) and xylazine (100 mg/mL) at a concentration of 0.6 mL/kg body weight. Using sterile technique, an 18 Ga x 15 cm catheter (SA1815; Mila International, Inc., Florence, KY, USA) was inserted into the right jugular vein and sutured to the skin using 2 – 0 monofilament suture and an extension attached.

At the time of catheter placement, an ultrafiltration probe (Canine UF Probe, BASi systems, W. LaFayette, IN, USA) was placed subcutaneously along the epaxial muscles using a previously described technique (28). The interstitial probe allowed for continuous collection of interstitial fluid (ISF). Pigs were able to recover for 36–48 hours following the placement of instrumentation. During this recovery period, patency of the catheter was maintained by removing the heparin lock (100 mg/mL), flushing the catheter with saline and replacing the heparin lock every 12 hours.

5.3 Drug Administration and Sample Collection

Pigs were administered a single intramuscular dose of either 0.4 mg/kg meloxicam (Meloxicam solution for injection 5 mg/mL, Putney, Inc., Portland, ME, USA), the labeled dose for pigs in Europe, or 2.2 mg/kg flunixin meglumine (Banamine-S®, Merck Animal Health, Summit, NJ, USA), the labeled dose for pigs in the US. Blood samples (3 mL) were collected via the jugular catheter and transferred to lithium heparinized tubes at 0 (baseline), 0.08, 0.17, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 132, 156 and 180 h post-administration of flunixin or meloxicam. Blood samples were centrifuged at 3500 x g and the plasma collected for analysis of total drug concentrations.

Interstitial fluid samples were collected via the preplaced collection probes at 0 (baseline), 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 132, 156 and 180 h post-dose and weighed to determine the volume collected. At the end of the experiment, the ISF probe was removed and the tubing length measured. A lag time for the ISF collection was calculated to account for the time taken for the sample to travel along the ISF probe tubing. Interstitial fluid was used to quantify the free (protein unbound/pharmacologically active portion) drug concentrations in the tissues.

In order to determine drug concentrations in urine, animals were housed individually in metabolism cages to allow for collection of urine samples. Urine samples were collected at 0 (pretreatment), 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 h after drug administration. Urine was collected via a tray under the metabolism cages with a spout at the front of the cage and a stainless-steel bucket underneath to catch the urine and limit fecal contamination. Further details of the sample collection and calculation are described in our previous study in goats by Bublitz et al (2019) (2).. All plasma, ISF and urine samples were frozen at -80 ºC prior to analysis

5.4 Tissue collection

After two consecutive negative (drug-free) urine samples (36–72 h for meloxicam, and 96–168 h for flunixin), each pig was sedated with 50:50 ketamine:xylazine and then humanely euthanized using intravenous pentobarbital sodium and phenytoin sodium. Several biopsy punches were taken from each lobe of the liver and the entire left and right kidneys were taken from each pig in order to analyze tissues when the drug was no longer detectable in urine. These tissue samples were frozen at -20^{o}C until analysis.

5.5 Drug Analysis

5.5.1 Plasma and Urine Sample Preparation

Flunixin and meloxicam plasma and urine samples were prepared using solid-phase extraction prior to UPLC-MS/MS analysis. Samples (300 µL) were pretreated with 300 µL of 4% phosphoric acid and vortexed for 10 seconds. Then, 500 µL of this pretreated sample was loaded onto an Oasis 1 mL 30 mg PRiME HLB cartridge (Waters Corp.), washed with 1 mL of methanol, and eluted from the cartridge with 500 µL of 90:10 (vol/vol) acetonitrile:methanol. The eluate was filtered through a 0.2 µm PTFE Whatman Mini-UniPrep Syringeless Filter vial (GE Healthcare UK Limited., Buckinghamshire, UK) and then injected onto the UPLC-MS/MS system.

5.5.2 Tissue Sample Preparation

For all kidney and liver samples, a 0.1 g sub-sample was weighed into a 2-mL bead mill tube containing 2.8-mm ceramic beads (Fisher Scientific, Hampton, NH, USA). Then, 1 mL of acetonitrile was added to the tube and the contents homogenized 3 times for 15 seconds during each cycle at a speed of 5 m/s for kidney, and 4 m/s for liver, with a 10 second rest between cycles (BeadMill24, Fisher Scientific). Following homogenization, the tubes were centrifuged at 10,000 x g for 5 minutes. Then, 800 µL of supernatant was transferred to a 16 × 100 mm borosilicate glass tube containing 800 µL of acetonitrile and 400 µL of water. This mixture was vortexed gently for 10 seconds and then eluted through a 3 mL Captiva EMR-Lipid cartridge (Agilent Technologies, Inc., Santa Clara, CA, USA). The eluate was then evaporated to dryness at 55 °C for 25 minutes. The sample was reconstituted in 300 µL of 1:1 acetonitrile:water, vortexed for 30 seconds, and the contents transferred a 0.2 µm PTFE Whatman Mini-UniPrep Syringeless Filter vial and then injected onto the UPLC-MS/MS system.

5.5.3 UPLC-MS/MS Conditions

All samples were quantified by ultra-high-pressure liquid chromatography (UPLC) with mass spectrometric (MS/MS) detection (Waters Corp., Milford, MA, USA). The UPLC-MS/MS system consisted of a Xevo TQD tandem quadrupole mass spectrometer (Waters Corp.)

For flunixin samples, separation was achieved with a 2.1 mm x 100 mm, 1.7 µm Waters Acquity BEH Phenyl column (Waters Corp.). A gradient was used, and the initial mobile phase was 0.1% formic acid in water: 0.1% formic acid in acetonitrile (70:30 v/v) with a flow rate of 0.4 mL/min for the first 2.5 minutes. The mobile phase then switched to (10:90 v/v) from 2.5 min – 3.5 min. For the last 1.5 min of the run, the mobile phase was (70:30 v/v). The MS/MS was run in ESI + mode. The quantification trace used was 297 → 279. Column temperature was 35 °C and sample temperature was ambient.

For meloxicam samples, separation was achieved with a 2.1 mm x 50 mm, 1.7 um Waters Acquity BEH C18 column (Waters Corp.) A gradient was used, and the initial mobile phase was 0.1% formic acid in water: 0.1% formic acid in acetonitrile (65:35 v/v) with a flow rate of 0.4 mL/min for the first minute. The mobile phase then switched to (10:90 v/v) from 1.0 min – 1.1 min. For the last 1.9 min of the run, the mobile phase was (65:35 v/v). The MS/MS was run in ESI + mode. The quantification trace used was 352.043 → 115. Column temperature was 35 °C and sample temperature was 10 °C.

Validation standards were prepared over a linear range for each matrix (plasma, urine, kidney and liver) and were used to construct calibration curves. All calibration curves were linear with a R^2 value of 0.99 or higher.

Limit of quantification, inter-day accuracy and inter-day precision are presented in Table 3 for each analytical method.

Table 3
Limit of quantification (LOQ; g/mL for fluids or g/g for tissues), inter-day accuracy (%) and inter-day precision (%) for analytical methods. SD; standard deviation

Sample Analysis Parameters						
Drug	Tissue	LOQ	Accuracy (%)		Precision (%)	
		µg/mL or µg/g	Mean	±SD	Mean	±SD
Flunixin	Plasma	0.0005	107	(6)	5	(3)
	ISF	0.0005	99	(7)	6	(5)
	Urine	0.0005	103	(5)	6	(3)
	Liver	0.0001	100	(5)	5	(2)
	Kidney	0.0001	100	(7)	8	(5)
Meloxicam	Plasma	0.001	100	(5)	6	(5)
	ISF	0.001	97	(6)	6	(2)
	Urine	0.001	104	(5)	4	(2)
	Liver	0.005	100	(5)	7	(2)
	Kidney	0.005	100	(4)	8	(2)

5.6 Pharmacokinetic analysis

A noncompartmental analysis of drug plasma concentration vs. time profiles was performed with Phoenix WinNonLin software (version 8.0; Certara, Princeton, NJ, USA). The area under the plasma concentration–time curve from time zero to infinity (AUC0→∞; h*µg/mL) was calculated by the linear trapezoidal rule. The AUC0→∞ was used to calculate clearance per fraction absorbed (Cl/F; L/h/kg) and half-life (T1/2; h). The volume of distribution (per fraction absorbed) (Vd/F; L/kg) was also calculated. Peak concentration (Cmax; µg/mL) and time at which maximum concentration occurs (Tmax; h) in plasma and urine was taken directly from the data from each pig.

Individual renal clearance values corrected for body weight were estimated for each pig using the following equation: Renal Clearance (mL/h) = [(Ae/AUC) * BW]. Where Ae is the cumulative amount of drug excreted unchanged in the urine, AUC is the area under the plasma concentration-time curve to infinity and BW is the body weight of each individual pig (kg).

Abbreviations

EMA; European Medicines Agency

FDA; Food and Drug Administration

FSIS; Food Safety and Inspection Service

ISF; Interstitial fluid

LOQ; Limit of Quantification

MRL; Maximum residue limit

NSAID; Nonsteroidal anti-inflammatory drug

SD; Standard deviation

UPLC-MS/MS; Ultra performance liquid chromatography-tandem mass spectrometer

USDA; United States Department of Agriculture

Declarations

Ethics approval and consent to participate

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

Consent for publication

Not applicable

Availability of data and materials

Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Major Livestock Shows of Texas [Grant #07 486432-01001] and the Food Animal Residue Avoidance Databank, United States Department of Agriculture - National Institutes of Food and Agriculture [Grant #2017 41480-27309]. Neither funding body had any role in the study design, sample collection, analysis, interpretation or writing the manuscript.

Authors' contributions

EN contributed to study design, sample collection, sample analysis, pharmacokinetic and statistical analysis, and writing the manuscript. TPM contributed to study design and editing the manuscript. PAR contributed to study design and sample collection. JLY contributed to sample collection and sample analysis. VRF contributed to study design and editing the manuscript. TH contributed to study design and editing the manuscript. REB contributed to study design and editing the manuscript.

All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Riviere JE, Webb AI, Craigmill AL. Primer on estimating withdrawal times after extralabel drug use. J Am Vet Med Assoc. 1998;213(7):966–8.
2. Bublitz CM, Mzyk DA, Mays T, Fajt VR, Hairgrove T, Baynes RE. Comparative plasma and urine concentrations of flunixin and meloxicam in goats. Small Rumin Res. 2019;174(January):40–6.
3. Shelver WL, Smith DJ, Tell LA, Baynes RE, Schroeder JW, Riviere JE. Screening and Confirmatory Analyses of Flunixin in Tissues and Bodily Fluids after Intravenous or Intramuscular Administration to Cull Dairy Cows with or without Lipopolysaccharide Challenge. J Agric Food Chem. 2016 Jan 13;64(1):336–45.
4. Sidhu PK, Gehring R, Mzyk DA, Marmulak T, Tell LA, Baynes RE, et al. Avoiding violative flunixin meglumine residues in cattle and swine. Vol. 250, Journal of the American Veterinary Medical Association. American Veterinary Medical Association; 2017. p. 182–9.
5. FDA. Tolerances for residues of new animal drugs in food. 21 CFR 556.286.
6. Smith DJ, Shelver WL, Baynes RE, Tell L, Gehring R, Li M, et al. Excretory, Secretory, and Tissue Residues after Label and Extra-label Administration of Flunixin Meglumine to Saline- or Lipopolysaccharide-Exposed Dairy Cows. J Agric Food Chem. 2015 May 20;63(19):4893–901.
7. Wasfi IA, Boni NS, Abdel Hadi AA, Elghazali M, Zorob O, Alkatheeri NA, et al. Pharmacokinetics, metabolism and urinary detection time of flunixin after intravenous administration in camels. J Vet Pharmacol Ther. 1998;21(3):203–8.
8. Soma LR, Behrend E, Rudy J, Sweeney RW. Disposition and excretion of flunixin meglumine in horses. Am J Vet Res. 1988 Nov;49(11):1894–8.
9. Brady TC, Yang TJ, Hyde WG, Kind AJ, Hill DW. Detection of flunixin in greyhound urine by a kinetic enzyme-linked immunosorbent assay. J Anal Toxicol. 21(3):190–6.
10. Coetzee JF. A study to assess the correlation between plasma, oral fluid and urine concentrations of flunixin meglumine with the tissue residue depletion profile in finishing age swine – NPB #13-210. Res Rep Pork Checkoff [Internet]. 2015; Available from: https://www.pork.org/wp-content/uploads/2007/08/13-210-COETZEE-ISU.pdf
11. Yu Z, Luo X, Zhang Z, Peng L. Determination of flunixin in swine plasma, urine and feces by UPLC-MS/MS and its application in the real samples. Curr Pharm Anal. 2019;15:51–60.

12. Gans J, Mercer P. Chapter 37 The Kidneys. In: Swenson M, editor. Duke's Physiology of Domestic Animals, 9th edition. Ithaca, New York: Cornell University Press; 1977. p. 463–92.
13. Toutain PL, Reymond N, Laroute V, Garcia P, Popot MA, Bonnaire Y, et al. Pharmacokinetics of meloxicam in plasma and urine of horses. Am J Vet Res. 2004 Nov;65(11):1542–7.
14. EMEA. Committee for Veterinary Medicinal Products Flunixin Summary Report (1) [Internet]. 1999 [cited 2019 Nov 17]. Available from: http://www.eudra.org/emea.html
15. United States Department of Agriculture. Title: Screening and Confirmation of Animal Drug Residues by UHPLC-MS-MS [Internet]. 2018 [cited 2019 Oct 28]. Available from: https://www.fsis.usda.gov/wps/wcm/connect/b9d45c8b-74d4-4e99-8eda-5453812eb237/CLG-MRM1.pdf?MOD=AJPERES
16. EMEA. Committee for Veterinary Medicinal Products Meloxicam (Extension to pigs) [Internet]. 2000 [cited 2019 Nov 17]. Available from: http://www.eudra.org/emea.html
17. Pairis-Garcia MD, Karriker LA, Johnson AK, Kukanich B, Wulf L, Sander S, et al. Pharmacokinetics of flunixin meglumine in mature swine after intravenous, intramuscular and oral administration. BMC Vet Res. 2013 Aug 13;9:1–7.
18. Nixon E, Almond GW, Baynes RE, Messenger KM. Comparative plasma and interstitial fluid pharmacokinetics of meloxicam, flunixin and ketoprofen in neonatal piglets. Front Vet Pharmacol. 2019. Manuscript in press.
19. Levionnois OL, Fosse TK, Ranheim B. PK/PD modeling of flunixin meglumine in a kaolin-induced inflammation model in piglets. J Vet Pharmacol Ther. 2018 Apr 1;41(2):314–23.
20. Mzyk DA, Bublitz CM, Hobgood GD, Martinez MN, Smith GW, Baynes RE. Effect of age on the pharmacokinetics and distribution of tulathromycin in interstitial and pulmonary epithelial lining fluid in healthy calves. Am J Vet Res. 2018 Nov 1;79(11):1193–203.
21. Buur JL, Baynes RE, Smith G, Riviere JE. Pharmacokinetics of flunixin meglumine in swine after intravenous dosing. J Vet Pharmacol Ther. 2006 Oct 1;29(5):437–40.
22. Viscardi AV. Evaluation of Analgesia Efficacy in Piglets Undergoing Surgical Castration and Tail Docking [Internet]. The University of Guelph; 2018 [cited 2019 Aug 29]. Available from: https://atrium2.lib.uoguelph.ca/xmlui/bitstream/handle/10214/13041/Viscardi_Abbie_201805_PhD.pdf?sequence=3&isAllowed=y
23. Fosse TK, Spadavecchia C, Horsberg TE, Haga HA, Ranheim B. Pharmacokinetics and pharmacodynamic effects of meloxicam in piglets subjected to a kaolin inflammation model. J Vet Pharmacol Ther. 2011 Aug 1;34(4):367–75.
24. Pairis-Garcia MD, Johnson AK, KuKanich B, Wulf L, Millman ST, Stalder KJ, et al. Pharmacokinetics of meloxicam in mature swine after intravenous and oral administration. J Vet Pharmacol Ther. 2015 Jun 1;38(3):265–70.
25. Fosse TK, Haga HA, Hormazabal V, Haugejorden G, Horsberg TE, Ranheim B. Pharmacokinetics and pharmacodynamics of meloxicam in piglets. J Vet Pharmacol Ther [Internet]. 2008 Jun 1 [cited 2019 Aug 29];31(3):246–52. Available from: http://doi.wiley.com/10.1111/j.1365-2885.2008.00958.x

26. Leemann T, Kondo M, Zhao J, Transon C, Bonnabry P, Dayer P. [The biotransformation of NSAIDs: a common elimination site and drug interactions]. Schweiz Med Wochenschr. 1992 Dec 5;122(49):1897–9.
27. Gasthuys E, Devreese M, Millecam J, Sys S, Vanderperren K, Delanghe J, et al. Postnatal maturation of the glomerular filtration rate in conventional growing piglets as potential juvenile animal model for preclinical pharmaceutical research. Front Pharmacol. 2017;8(JUN).
28. Messenger KM, Papich MG, Blikslager AT. Distribution of enrofloxacin and its active metabolite, using an in vivo ultrafiltration sampling technique after the injection of enrofloxacin to pigs. J Vet Pharmacol Ther. 2012 Oct;35(5):452–9.

Figures

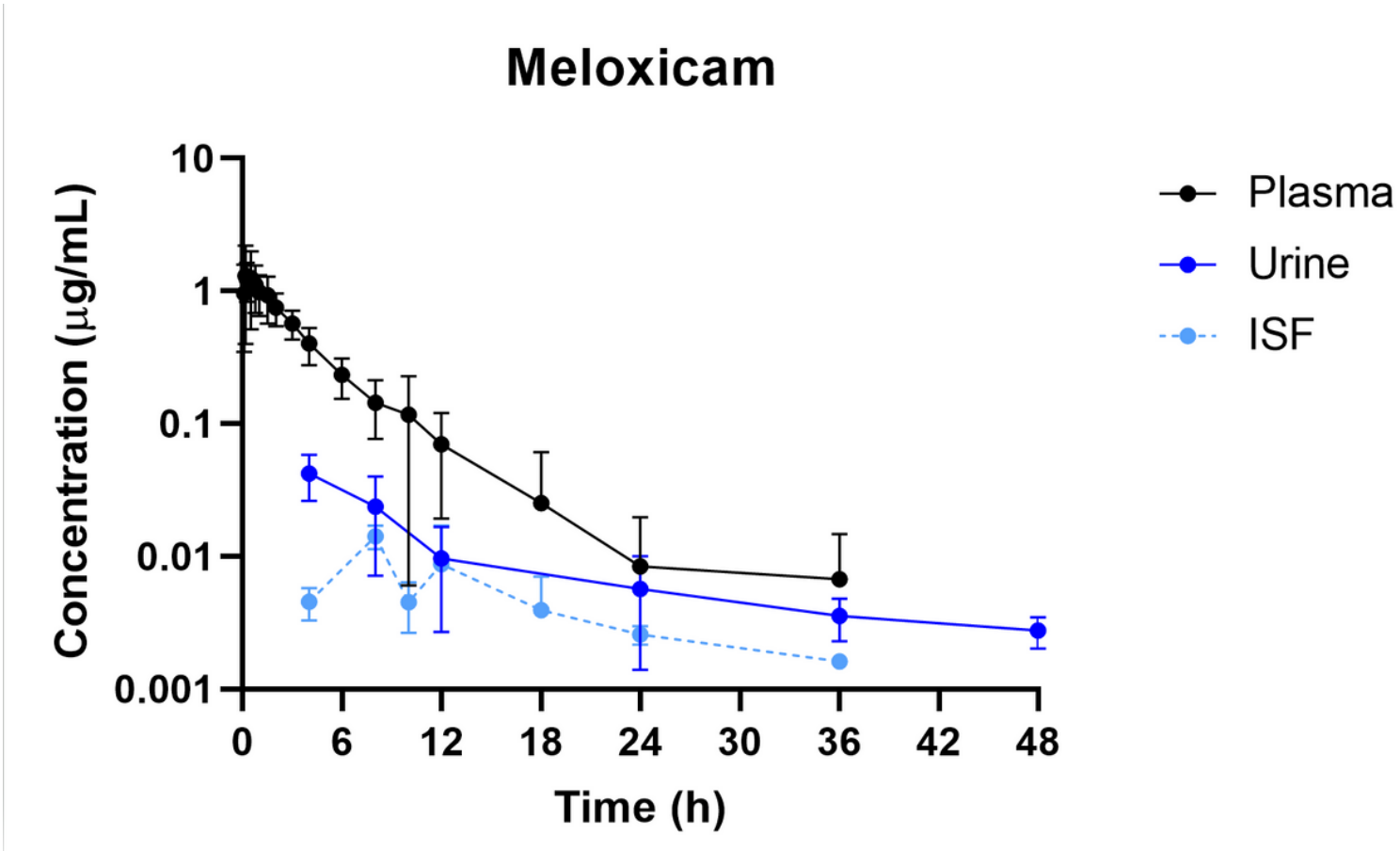

Figure 1

Plasma, urine and interstitial fluid meloxicam concentration-time profiles following intramuscular administration of 0.4 mg/kg meloxicam to pigs. Data are represented as mean ± standard deviation.

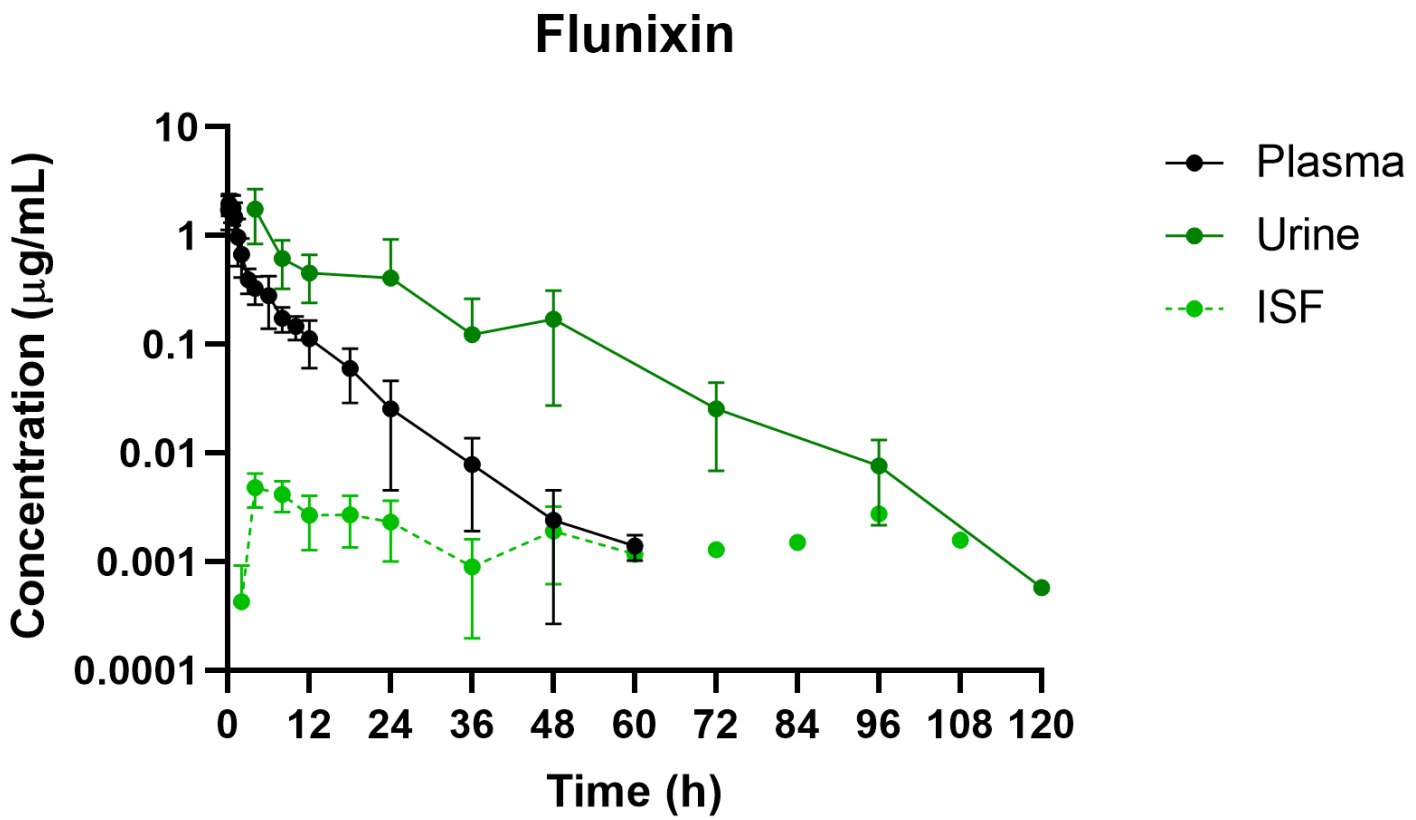

Figure 2

Plasma, urine and interstitial fluid flunixin concentration-time profiles following intramuscular administration of 2.2 mg/kg flunixin to pigs. Data are represented as mean ± standard deviation. Interstitial fluid concentrations shown at 72-108h are for a single pig only.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Checklist.pdf