Published in final edited form as:

J Vet Pharmacol Ther. 2012 August ; 35(4): 413–416. doi:10.1111/j.1365-2885.2011.01356.x.

Pharmacokinetics of Concurrently Administered Intravenous Lidocaine and Flunixin in Healthy Horses

Sarah J. Waxman¹, Butch KuKanich^{2,*}, Melissa Milligan³, Warren L. Beard⁴, and Elizabeth G. Davis⁴

¹Veterinary Clinical Sciences, Purdue University, 625 Harrison St., West Lafayette, IN 47907

²Department of Anatomy and Physiology, Kansas State University, College of Veterinary Medicine, Manhattan, KS 66506

³Equine Specialty Hospital, 17434 Rapids Rd., Burton, OH 44021

⁴Department of Clinical Sciences, Kansas State University, College of Veterinary Medicine, Manhattan, KS 66506

Lidocaine is a moderately protein bound drug often administered systemically in post-operative horses to treat symptoms of visceral pain and ileus (Milligan *et al.*, 2007; Nolen-Walston *et al.*, 2007). Flunixin is a highly protein bound drug non-steroidal anti-inflammatory drug (NSAID) also administered to horses to treat visceral pain and inflammation associated with colic (Coakley *et al.*, 1999; Pellegrini-Masini *et al.*, 2004; Moore, 2006). Therefore, these drugs are often used in combination to treat horses. The pharmacokinetics of these drugs have been examined independently in the horse (Coakley *et al.*, 1999; Pellegrini-Masini *et al.*, 2004; Milligan *et al.*, 2006), but to the authors' knowledge, they have not been examined while administered concurrently in the horse. A previous study (Milligan *et al.*, 2006) found potentially meaningful *in vitro* interactions in the ultrafiltrate concentrations of lidocaine when these drugs were combined. The purpose of this study was to determine lidocaine plasma and plasma ultrafiltrate concentrations and flunixin plasma concentrations when concurrently administered to healthy horses.

Six horses were included ranging 13.5 to 24.5 years in age. Horses were healthy based on a physical exam, complete blood count, and serum chemistry panel. Horses were confined to a stall for the study with access to brome hay and water. The Institutional Animal Care and Use Committee at Kansas State University approved the study.

Two jugular catheters were placed in each animal, the left jugular catheter dedicated for drug administration, the right jugular catheter for sampling. Lidocaine skin blocks were performed prior to placing catheters. Lidocaine was administered as a loading dose (1.3 mg/kg over 15 minutes) and then a constant rate infusion (50 µg/kg/min) for 6 hours via a fluid pump. Flunixin meglumine (Banamine, Schering-Plough Animal Health, Union, NJ, USA) (1.1 mg/kg as flunixin) was administered by a bolus injection 2 hours after initiating lidocaine administration. Blood samples, 9 mL each, were acquired at 0, 1, 1.5, 2, 2.25, 2.5,

*Corresponding Author kukanich@ksu.edu, 785-532-4557.

2.75, 3, 4, 6, and 8 hours after lidocaine administration. Blood was immediately centrifuged after collection at $3000 \times g$ at ambient temperature for 5 minutes with the resulting plasma aliquotted for ultrafiltration or storage at -70°C until analysis. The plasma ultrafiltrate was immediately obtained after 35 minutes of centrifugation of the plasma at ambient temperature using a 10,000 MW ultrafiltration device (Amicon Centrifree, Millipore Corp., Bedford, MA, USA). It is important to iterate that the plasma ultrafiltrate may not truly represent *in vivo* unbound plasma concentrations as temperature may affect the degree of plasma protein binding. The temperature was not maintained at normal equine body temperature during processing of plasma and ultrafiltrate due to lack of heated centrifuges. However the effects of temperature have not been specifically evaluated for lidocaine in equine plasma.

Total plasma lidocaine and plasma ultrafiltrate lidocaine concentrations were determined using high-pressure liquid chromatography (HPLC)-mass spectrometry (LCQ DUO, Thermo Electron Corporation, Waltham, MA, USA) and flunixin plasma concentrations were determined using HPLC (Finnigan Spectrasystem, Thermo Electron Corporation, Waltham, MA, USA). Lidocaine and flunixin were extracted from plasma with solid phase extraction cartridges (Bond Elut C8; Varian Inc., Palo Alto, CA, USA). Cartridges were conditioned with 1 mL methanol and 1 mL distilled water. One mL of plasma (or 0.1 mL plasma ultrafiltrate) was then loaded, washed with 1 mL distilled water, and eluted with 1 mL methanol. Samples were evaporated to dryness at 40°C under nitrogen and reconstituted with 200 μL of 50% methanol. The reconstituted samples were centrifuged using a $0.45 \mu\text{M}$ filter device (Costar, Spin-X, Corning Inc., Corning, NY, USA) to remove particulates.

The internal standard for lidocaine was bupivacaine. Lidocaine standard curves were generated daily by fortifying untreated equine plasma (or plasma ultrafiltrate) with lidocaine (Spectrum Chemical, Gardena, CA, USA) and accepted if the coefficient of determination (R^2) was at least 0.99 and the predicted concentrations were within 15% of the actual concentrations (Table 1). The injection volume was 15 μL for plasma and 75 μL for ultrafiltrate.

The internal standard used for flunixin was indoprofen. Flunixin standard curves were generated daily by fortifying untreated equine plasma with flunixin (Spectrum Chemical, Gardena, CA, USA) and accepted as described for lidocaine (Table 1). The injection volume was 50 μL .

Total plasma lidocaine concentrations consisted of mean values from 1.37- 1.78 $\mu\text{g/mL}$ during the infusion (1-6 hours), which are within the expected range of concentrations (Fig. 1) (Milligan, *et al.*, 2006). Mean total concentrations of lidocaine decreased to 0.09 $\mu\text{g/mL}$ by two hours post termination of lidocaine CRI. Total body clearance (Table 2, 3) was calculated using total lidocaine concentrations, assumed to be at steady state and was within previously reported ranges (Milligan, *et al.*, 2006). Significant differences in total plasma lidocaine concentrations occurred at 3 hours ($P<0.05$) as compared to the 2.0 - 2.75 h samples, but was not significantly different from other time points during the infusion (Figure 1) using a one-way repeated measures analysis of variance (SigmaStat 3.1; Systat

Software Inc., Point Richmond, CA, USA). Similar changes in total plasma lidocaine concentrations during a CRI have been observed previously (Milligan et al., 2006).

The mean plasma ultrafiltrate lidocaine concentrations were 1.02-1.29 µg/mL during the infusion. No significant differences in the plasma ultrafiltrate concentrations occurred during the infusion (Figure 2). It is unknown if changes in ultrafiltrate lidocaine concentrations occurred earlier than 15 minutes after flunixin administration, as it was the first time point assessed. The amount of lidocaine present in equine plasma ultrafiltrate is greater than expected based on a previous *in vitro* study (Milligan et al., 2006). The reason for the discrepancy is unclear, but could include variations from *in vitro* and *in vivo* conditions, previous plasma storage in the *in vitro* study, or random variability. It is important to reiterate that plasma and ultrafiltrate processing were not conducted at normal equine body temperature; therefore they may not truly represent unbound or free drug concentrations in the animal. Further studies are needed directly comparing *in vitro* and *in vivo* plasma ultrafiltrate concentrations of lidocaine using the same animals, the effects of temperature during processing on equine ultrafiltrate drug concentrations, and assessing the effects of plasma storage on *in vitro* plasma ultrafiltrate concentrations.

The disposition of flunixin was as expected after administration of this dosage and the pharmacokinetics were similar to previously reported values for the same dosage (Pellegrini-Masini *et al.*, 2004), (Fig. 3, Table 4).

The study design was tolerated well by five of the six horses. One horse (13.5 year old Quarter Horse gelding with documented Hyperkalemic Periodic Paralysis [HYPP]) developed clinical signs consistent with an HYPP episode approximately 8.5 hours after initiation of lidocaine, which was 2.5 hours after discontinuation of the lidocaine infusion and one half hour after the final recorded data point. The horse was treated with intravenous calcium borogluconate and dextrose and recovered with no complications. It is unclear if this episode was triggered by the drug protocol, procedures associated with the study, or if it was a random episode. None of this animal's data are excluded from the study.

Flunixin (1.1 mg/kg) was administered as a bolus 2 hours after initiation of lidocaine treatment to allow lidocaine to reach steady state concentration, and to have baseline data in these horses. Despite administration of flunixin no changes in plasma ultrafiltrate lidocaine concentrations occurred, but significant differences in total lidocaine concentrations occurred at a single time point, 3 hours (1 hour after flunixin). It is unclear if the significant difference in the total lidocaine concentration was due to flunixin, potential variability in lidocaine infusion, altered lidocaine clearance, or random variability. However, the variability of the infusion was minimized by using a fluid pump.

Total plasma lidocaine concentrations were within the expected range reported for treating postoperative ileus in the horse (Malone *et al.*, 2006). Baseline (T=0) lidocaine concentrations were detected due to absorption of lidocaine used as a skin block approximately 30 minutes prior to catheter placement. Although this does not affect the study, it is an indication that detectable plasma concentrations of lidocaine are achieved following the local administration of lidocaine.

Flunixin concentrations were within the range expected in healthy horses (Pellegrini-Masini *et al.*, 2004) (Table 4). All pharmacokinetic variables were similar to the previous study except for C_0 (concentration extrapolated to time zero) with a geometric mean of 10.88 $\mu\text{g/mL}$ (range 8.04 – 13.7 $\mu\text{g/mL}$), versus 24.7 $\mu\text{g/mL}$ in the referenced study. The lower C_0 in this study could be because our first blood sample was obtained at 15 minutes after flunixin administration, while the first blood sample obtained in the referenced study was at 5 minutes after drug administration. C_0 is determined by regression of the first 2 plasma concentrations; therefore the timing of the initial samples will affect the estimated C_0 . It is also possible that the potential difference in C_0 may just be due to random variability in horses.

In conclusion, plasma lidocaine concentrations and plasma flunixin concentrations remained within their expected ranges when administered concurrently in the healthy horse (Meyer *et al.*, 2001). Further investigation is required to establish interactions of these drugs in unhealthy horses.

Acknowledgments

The authors would like to thank the Grayson-Jockey Club Research Foundation, Kansas State University College of Veterinary Medicine, the Department of Anatomy and Physiology at Kansas State University, and the Analytical Pharmacology Laboratory at Kansas State University for providing financial support. We would also like to thank the Veterinary Research Scholars Symposium at Kansas State University for providing support for this study through NIH T35RR007064. We would also like to thank the Morris Animal Foundation Veterinary Student Scholars Program for providing support.

References

- Coakley M, Peck KE, Taylor TS, Matthews NS, Mealey KL. Pharmacokinetics of flunixin meglumine in donkeys, mules, and horses. *American Journal of Veterinary Research*. 1999; 60(11):1441–1444. [PubMed: 10566824]
- Dickey EJ, McKenzie HC 3rd, Brown KA, de Solis CN. Serum concentrations of lidocaine and its metabolites after prolonged infusion in healthy horses. *Equine Veterinary Journal*. 2008; 40:348–352. [PubMed: 18267881]
- Engelking LR, Blyden GT, Lofstedt J, Greenblatt DJ. Pharmacokinetics of antipyrine, acetaminophen and lidocaine in fed and fasted horses. *Journal of Veterinary Pharmacology and Therapeutics*. 1987; 10:73–82.
- Feary DJ, Mama KR, Wagner AE, Thomasy S. Influence of general anesthesia on pharmacokinetics of intravenous lidocaine infusion in horses. *American Journal of Veterinary Research*. 2005; 66:574–580. [PubMed: 15900935]
- Malone E, Ensink J, Turner T, Wilson J, Andrews F, Keegan K, Lumsden J. Intravenous continuous infusion of lidocaine for treatment of equine ileus. *Veterinary Surgery*. 2006; 35(1):60–66. [PubMed: 16409411]
- Meyer GA, Lin HC, Hanson RR, Hayes TL. Effects of intravenous lidocaine overdose on cardiac electrical activity and blood pressure in the horse. *Equine Veterinary Journal*. 2001; 33(5):434–437. [PubMed: 11558736]
- Milligan M, Beard W, Kukanich B, Sobering T, Waxman S. The effect of lidocaine on postoperative jejunal motility in normal horses. *Veterinary Surgery*. 2007; 36(3):214–220. [PubMed: 17461945]
- Milligan M, Kukanich B, Beard W, Waxman S. The disposition of lidocaine during a 12-hour intravenous infusion to postoperative horses. *Journal of Veterinary Pharmacology and Therapeutics*. 2006; 29(6):495–499. [PubMed: 17083453]
- Moore, RM. Principles of Medical Management of Colic; Principles of Medical Management of Colic North American Veterinary Conference; Orlando, FL. 2006.

- Nolen-Walston R, Paxson J, Ramey DW. Evidence-based gastrointestinal medicine in horses: it's not about your gut instincts. *Veterinary Clinics of North America Equine Practice*. 2007; 23(2):243–266.
- Pellegrini-Masini A, Poppenga RH, Sweeney RW. Disposition of flunixin meglumine injectable preparation administered orally to healthy horses. *Journal of Veterinary Pharmacology and Therapeutics*. 2004; 27(3):183–186. [PubMed: 15189304]

Figure 1.

Mean $\pm$ standard deviation concentration of total plasma lidocaine at each sampling time point during infusion of lidocaine (50 $\mu\text{g/kg/min}$). The infusion was terminated at 6 hours. A lidocaine skin block was performed prior to placement of jugular catheters. The asterisk indicates the time point (3 hour) which was significantly different from the 2.0 h ($P=0.021$), 2.25 h ($P=0.004$), 2.5 h ($P=0.038$), and 2.75 h ($P=0.009$) post administration samples. The arrow indicates the time flunixin was administered.

Figure 2.

Mean $\pm$ standard deviation concentration of lidocaine in plasma ultrafiltrate at each sampling time point during infusion of lidocaine (50 $\mu\text{g/kg/min}$). No significant differences occurred during the infusion. The infusion was terminated at 6 hours. A lidocaine skin block was performed prior to placement of jugular catheters. The arrow indicates the time flunixin was administered.

Figure 3.

Mean $\pm$ standard deviation concentration of plasma flunixin at each sampling time point after intravenous administration of 1.1 mg/kg. Flunixin was administered 2 hours post initiation of lidocaine administration. The arrow indicates the time flunixin was administered.

Waxman et al.

Page 9

Characteristics of the analytical assays for lidocaine and flunixin. The accuracy and precision of the lidocaine were determined on replicates of 4 at each of the following concentrations: 0.05, 0.25, 0.5, and 2.5 µg/mL. The accuracy and precision of the flunixin method were determined on replicates of 4 at each of the following concentrations: 1.25, 2.5, 12.5, 25 µg/mL. Mobile phase A=0.1% trifluoroacetic acid in water; mobile phase B=acetonitrile. A 150 × 3.0 mm, 5 µm C18 column achieved separation of lidocaine and flunixin (Zorbax XDB; Agilent Technologies, Palo Alto, CA, USA).

Table 1

	Linear range (µg/mL)	Lower Limit of quantification (µg/mL)	Accuracy	Coefficient of variation	Mobile Phase	Quantification
Lidocaine	0.05 – 2.5	0.05	108%	8%	0-8 min=90%A:10 B 8-12 min=50%A:50%B 12-14 min=90%A : 10%B Flow rate = 0.3 mL	MS/MS Lidocaine: <i>m/z</i> 235 →86 Bupivacaine, IS: <i>m/z</i> 289 → 140
Flunixin	0.625 - 25	0.625	101%	5%	55%A:45% B Flow rate = 0.3 mL	UV 254 nM

Table 2

Calculated total body clearance of lidocaine at each time point assuming the plasma concentrations were at steady state. Total body clearance was calculated using the equation: $Cl = \text{dose} \div C_{\text{Steady State}}$. Panel B:

Previously published values of lidocaine clearance in healthy horses.

Time (hrs)	Mean (mL/min/kg) $\pm$ SD	Minimum	Maximum
1	32.98 $\pm$ 7.07	24.52	42.85
1.5	33.62 $\pm$ 7.60	24.44	41.86
2	35.87 $\pm$ 6.36	28.58	43.45
2.25	37.09 $\pm$ 5.28	28.37	44.51
2.5	35.64 $\pm$ 7.37	26.16	46.95
2.75	36.46 $\pm$ 6.21	29.18	41.70
3	29.24 $\pm$ 6.29	20.52	37.31
4	30.86 $\pm$ 5.93	22.87	37.78
6	32.80 $\pm$ 6.33	26.58	37.70

Table 3

Previously reported clearance of lidocaine in healthy horses.

Reference	Mean (mL/min/kg) $\pm$ SD
Engelking et al., 1987	43 $\pm$ 9, 52 $\pm$ 12
Feary et al, 2005	29 $\pm$ 8
Milligan et al., 2006	16 $\pm$ 3 – 30 $\pm$ 5
Dickey et al., 2008	49 - 55

Table 4

Pharmacokinetic values via noncompartmental analysis for flunixin administered intravenously at 1.1 mg / kg for this study as compared to Pellegrini-Masini *et al.*, 2004.

Variable	Geometric Mean (range)	Geometric Mean (Pellegrini-Masini <i>et al.</i> , 2004)
C ₀ (µg/mL)	10.88 (8.04-13.7)	24.7
Cl (mL/min/kg)	0.63 (0.48-0.81)	0.517
t _{1/2λ} (Hr)	1.92 (1.64-2.14)	2.1
V _z (L/kg)	0.100 (0.081-0.138)	0.10