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Pharmacokinetics of intravenous, subcutaneous, and topical administration of lidocaine hydrochloride and metabolites 3-hydroxylidocaine, monoethylglycinexylidide, and 4-hydroxylidocaine in the horse.

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Abbreviations

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- 25 K_e = elimination rate constants
- 26 k = fractional rate constants
- 27 $t_{1/2e}$ = elimination half-life
- 28 K_{ab} = absorption rate constant
- 29 $t_{1/2ab}$ = absorption half-life
- 30 A, B, C = coefficients
- 31 α, β, γ = slope factor or exponent
- 32 $t_{1/2\alpha}, t_{1/2\beta}, t_{1/2\gamma}$ = α, β, γ half-lives
- 33 V_C, V_2, V_3 = volumes of compartments
- 34 V_{ss} = volume of distribution at steady state
- 35 Cl_C = compartmental clearance
- 36 AUC_0^{lst} = area under the concentration-time curve
- 37 T_{max} = time to maximum concentration;
- 38 C_{max} = maximum concentration

39

40 ABSTRACT

41 Intravenous (iv), subcutaneous (sq), and topical (tp) lidocaine was administered to six horses in a
 42 cross-over, randomized design study. Samples were collected for up to 72 h. Compartmental
 43 models were used to investigate the pharmacokinetics of (LD) and its metabolites 3-
 44 hydroxylidocaine (3-OH), 4-hydroxylidocaine (4-OH), and monoethylglycinexylidide (MEGX).
 45 Metabolites 3-OH and 4-OH were present in conjugated forms, whereas LD and metabolite
 46 MEXG were present primarily in the un-conjugated form. Plasma concentrations of LD after iv

administration (100 mg) were described by 3-compartment model with an additional 3 compartments to describe the elimination of metabolites. Median (range) elimination micro-constants (K_e) for LD, 3-OH, 4-OH, and MEXG were 4.12(2.62-6.23), 1.25(1.10-2.15), 1.79(1.22-2.39), and 1.69(1.03-1.99) /h, respectively. Median (range) values of alpha ($t_{1/2\alpha}$), beta ($t_{1/2\beta}$), and gamma ($t_{1/2\gamma}$) half-lives were 0.08(0.07-0.13), 0.57(0.15-1.25), and 4.11(0.52-7.36) h. Plasma concentrations of LD after sq (200 mg) administration were described by absorption and 2-compartment elimination model. The median (range) of the LD absorption half-life ($t_{1/2ab}$) was 0.47(0.29-0.61) h. The K_e for LD, 3-OH, 4-OH, and MEXG were 3.91 (1.48-9.25), 1.00 (0.78-1.08), 1.76 (0.96-2.11), and 1.13(0.69-1.33) /h. The median (range) of $t_{1/2\alpha}$ and $t_{1/2\beta}$ were 0.15 (0.06-0.27) and 3.04 (2.53-6.39) h. Plasma concentrations of LD after tp (400 mg) application were described by 1-compartment model with a $t_{1/2ab}$ of 8.49(5.16-11.80) h. The K_e for LD, 3-OH and MEXG were 0.24 (0.10-0.81), 0.41 (0.08-0.93), and 0.38(0.26-1.14)/h.

INTRODUCTION

Lidocaine hydrochloride (LD) is extensively used in veterinary practice (Heavner, 1981; Webb & Pablo, 2009; Hubbell, 2010). It is commonly used for local analgesia, as a line block, blocking a specific nerve or group of nerves, intra-articular injection (Derksen, 1980; Dyson, 1993; Spoormakers, Donker et al., 2004) and regionally by epidural or ganglionic blockage (Schelling & Klein, 1985; Skarda, Muir et al., 1987). Topical preparations are by direct application (Rowbotham, Davies et al., 1995; Argoff, 2002) or LD patch (Argoff, 2002; Gammaioni, Alvarez et al., 2003; Ko, 2007; Ko, Maxwell et al., 2008).

Lidocaine has been administered intravenously by continuous infusion for the treatment of cardiac arrhythmias, ileus, and as an adjunct to general anesthesia (Malone, Ensink et al., 2006; Navas de Solis & McKenzie, 2007; Rezende, Wagner et al., 2011; Wagner, Mama et al., 2011)

Lidocaine is considered intermediate in its duration of action (~90 min.) with a fast onset and a potency two times that of procaine (Luduena, 1969; Webb & Pablo, 2009) and the duration

of analgesia is approximately 100 min (Tucker & Mather, 1979). The use of LD with epinephrine will prolong the analgesia (Spoormakers, Donker et al., 2004) by delaying its absorption (Braid & Scott, 1965).

Lidocaine is an amide with a structural formula of 2-(diethylamino)-N-(2, 6-dimethylphenyl) acetamide. A number of metabolites of have been described in the elimination of LD in horses; 3-hydroxylidocaine (3-OH), 4-hydroxylidocaine (4-OH), 4-hydroxy-2, 6-dimethylalanine (4-OH-DMA), and monoethylglycinexylidide (MEGX), 4-OH-monoethylglycinexylidide (OH-MEGX), 2-6-dimethylalanine (DMA), 4-hydroxylidocaine (4-OH), and glycine xylidide (GX) (Chalmers, Elgar et al., 1985; Crone, 1987; Short, Flory et al., 1988; Dirikolu, Lehner et al., 2000; Maes, Weiland et al., 2007; Navas de Solis & McKenzie, 2007; Nelis, Sievers et al., 2010; Scarth, Teale et al., 2011). Similar metabolites have been identified in the rat, guinea pig, dog, and man, with variation between species in the recovery of various metabolites (Keenaghan & Boyes, 1972).

This paper describes the pharmacokinetics and elimination of LD following iv, sq, and tp administration in horses by determining the plasma and urinary concentration of lidocaine (LD) (234.34 g/mol) and three primary metabolites 3-hydroxylidocaine (3-OH) (250.34 g/mol), 4-hydroxylidocaine (4-OH) (250.34 g/mol), monoethylglycinexylidide (MEGX) (206.28 g/mol) (Harkins, Mundy et al., 1998).

MATERIAL AND METHODS:

Horses

The study was approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Six Thoroughbreds (3 mares and 3 geldings) weighing $572.5 \pm \text{SD of } 53.5$ kg, ranging in age from 3 to 13 years were selected from the research herd. The horses were no longer actively racing but were otherwise in good health; routine foot, dental care, vaccination and deworming were performed on a scheduled basis and were housed on pasture with shelters. The horses were brought into climate-controlled stalls 2 days before the experiment and remained for the duration of the study. Hay and water were available *ad libitum*. Horses were weighed on the morning before the start of each study.

Drug administration

Lidocaine was administered to horses in a cross-over, randomized design with a minimum of 2 weeks elapsing between administrations. Five ml (100 mg) (0.17 ± 0.01 mg/kg) of a 2% solution

(Hospira Inc. IL, USA) of LD was administered as iv bolus. The sq injection 10 ml (200 mg) (0.35 ± 0.03 mg/kg) using a 22-gauge needle was centered over the right carpus. For the tp administration 8g of a 5% LD (400 mg) ointment was weighted and applied (0.68 ± 0.06 mg/kg) to a clipped carpus region (7.5 x 10 cm) and covered by a Tefla non-adherent dressing pad, (Tyco Healthcare U.K), the pad was wrapped with 4" elastic tape (Elastikon, Johnson and Johnson, NJ, USA) and left on for 24 hours. Lidocaine remaining on the skin surface was not removed from the carpal area and the collection of samples continued for 72 h. The LD ointment was in a soluble base containing water, ethoxydiglycol, trolamine, carbomer, allantoin, DMDM hydantoin, iodopropynyl butylcarbamate, ethyl aminobenzoate. (Taro Pharmaceuticals Inc. NJ, USA).

All blood samples were collected in Na fluoride/K Oxalate anticoagulant (Johnson and Johnson, NJ, USA) using a 14-gauge catheter (Angiocath, Becton Dickinson, UT, USA) placed into the jugular vein. For the iv administration two catheters were aseptically placed, one for injection and the second for contralateral collection of blood samples.

Blood samples were collected before drug administration (0 h) and at 2, 5, 10, 15, 20, 30, and 45 minutes and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 48, and 72 h post-drug administration. Heparinized saline and waste blood was withdrawn from the catheter into a 20 ml syringe too insure that undiluted blood was being collected; following the collection of the blood sample, the waste blood was reinjected, and the catheter flushed with heparinized saline. Blood samples were centrifuged (2,500 X g for 15 min) to harvest plasma. Three aliquots of two ml of plasma were immediately frozen and stored at -70°C until analyzed. The cross over study took four to five months, samples were stored at -70°C for no longer than the duration of the study.

Urine samples

In female horses for the 24-hours of continuous collection of urine, a sterile indwelling 24-F self-retaining catheter (Foley Catheters, CR Bard Inc., GA, USA) was aseptically placed in the bladder and attached to a drainage bag (Bard Center Entry Urinary Drainage Bag, CR Bard Inc., GA, USA). Urine samples were collected at 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 48 and 72 h after drug administration. In male horse a urine catcher was used. Urine samples (12 mL) were also stored at -70°C until analyzed. The volume was recorded for all urine samples collected during the first 24 h. Urine volume multiplied times analyte concentration was used to determine the total amount (μg) of LD and metabolites eliminated. Due to the differences in molecular

weights, micrograms were converted to moles for these calculations. Percent urinary elimination of each analyte was based on molar units of LD and metabolites relative to the total molar units eliminated.

Quantification of Lidocaine and its metabolites

Lidocaine hydrochloride was purchased from Sigma-Aldrich, MO, USA and 3-OH and 4-OH from Frontier BioPharm. KY, USA and MEXG from Alltech Associate-Applied Science, PA, USA. A sensitive analysis using ultrahigh-pressure liquid chromatography-tandem electrospray mass spectrometry (UHPLC-MS/MS) was developed and validated for quantification of lidocaine and its 3 primary metabolites in equine plasma and urine.

Samples Preparation. The effect of enzymatic hydrolysis on the quantification of lidocaine and its major metabolites was studied in the plasma samples from two horses collected after 1 h and 2 h lidocaine IV administration. The results showed that metabolites 3-OH and 4-OH lidocaine could not be detected in those plasma samples without enzymatic hydrolysis, suggesting that 3-OH and 4-OH lidocaine predominantly existed in conjugated form in plasma; LD and MEXG were not significantly affected by enzyme hydrolysis and presented primarily as free form in plasma (see Supplementary Table 1). Therefore, in this study, enzymatic hydrolysis was employed in sample preparation and the analytes quantified in this method included both conjugated and un-conjugated forms.

Plasma/urine samples were incubated with freshly prepared beta-glucuronidase at 65°C for 1.0 h to hydrolyze the sample and de-conjugated analytes presented as glucuronide conjugates. The hydrolyzed samples were adjusted to alkaline pH (10-11) and 5 mL of methyl *tert*-butyl ether was added to extract the analytes. The mixture was centrifuged (1300 x g for 10 min) and the top organic layer was evaporated to dryness at 60°C (TechniDri-Block DB-3, Duxford, Cambridge, UK) under a steady stream of air. The dried extract was reconstituted in 0.5 mL mobile phase. Twenty uL reconstituted sample was analyzed by UHPLC-MS/MS.

Liquid chromatography and mass spectrometry. For UHPLC-MS/MS analysis, analytes were separated on a Phenomenex Kinetex 1.7 μ m PFP column (50 x 2.1 mm) (Phenomenex, CA, USA) and analyzed by TSQ Quantum Ultra mass spectrometer (Thermo Fisher Scientific, CA, USA). Positive electrospray ionization mode was used with selected reaction monitoring (SRM). Mass spectrometer parameters common to all analytes were: spray voltage, 3500 v; vaporizer temperature, 205°C; sheath gas, 60 arbitrary units; ion sweep gas, 0 arbitrary units; auxiliary gas,

10 arbitrary units; ion transfer capillary temperature, 300°C; peak width relating to resolution (FWHM), 0.7 u for Q1 and Q3; collision gas pressure, 1.5 mTorr (1 Torr = 133 pa); scan width (m/z), 0.5; and scan time, 100 ms for each SRM scan. Data acquisition and analysis were accomplished by Xcalibur software v 2.0.7 (Thermo Fisher Scientific, CA, USA).

Quantification of lidocaine and its metabolites. The SRM transitions for quantification of lidocaine, 3-hydroxylidocaine, 4-hydroxylidocaine and MEXG were: m/z 235 $\rightarrow$ m/z 86, m/z 251 $\rightarrow$ m/z 86, m/z 251 $\rightarrow$ m/z 86, and m/z 207 $\rightarrow$ m/z 58, respectively. Deuterium-labeled lidocaine- d_{10} and 4-hydroxylidocaine- d_{10} were used as internal standards. The dynamic range was 0.1 to 100 ng/mL, with correlation coefficients (r) for the quadratic regression > 0.99 . Limits of quantification for LD, 3-OH, and 4-OH were 0.1 ng/mL and 0.5 ng/mL for MEXG. Method was validated based on FDA Guidance for Bioanalytical Industry (1) and the accuracy and precision at concentrations of 0.1, 0.5, 10, 50 and 100 ng/L determined (Supplementary Table 2). Standard operating procedures used by this laboratory meet requirements established by International Organization for Standardization. ISO/IEC 17025:2005: General requirements for the competence of testing and calibration laboratories www.iso.org/standard/39883.html. The laboratory is accredited by the American Association for Laboratory Accreditation (www.a2la.org).validation.<https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm368107>.

Pharmacokinetic Analysis

Compartmental analysis was used to describe the disposition and elimination of LD and its three primary metabolites 3-OH, 4-OH, and MEXG, following iv, sq, and tp administrations. Plasma concentration versus-time curves from each horse were analysed using conventional nonlinear least-squares regression analysis (Simulation, Analysis and Modeling Software, WinSaam.com.) Three and 2-compartment models were fitted to plasma concentrations of LD following iv and sq administrations and one-compartment model to tp application. The number of compartments required to best describe the LD and metabolite plasma concentrations was based on the reduction in the sums of squares, minimization of fractional standard deviation of each compartmental parameter, and the converging of the observed and predicted plasma concentrations curves.

A 3-compartment mammillary model with injection into and elimination from the central compartment (C_1), with inter-compartmental distribution rate constants to C_2 (k_{12} , k_{21}) and C_6

FOOTNOTE (1) U.S. Food and Drug Administration. Guidance for industry–Bioanalytical method

(k_{16} , k_{61}) were used to describe iv LD administration (**Figure 1a**). Additional compartments were added to incorporate the LD metabolites, where C_1 represented LD central compartment and C_3 , C_4 , and C_5 represented those of the three metabolites. The fractional rate constants (k_{13} , k_{14} , k_{15}) represent the transfer/metabolism from C_1 to respective compartments and (K_{10} , K_{30} , K_{40} , K_{50}) represent the elimination rate constants (K_e) from the central and respective compartments.

An absorption and 2-compartment elimination model was used to describe sq LD administration. Lidocaine was absorbed from the subcutaneous compartment (C_0) to the central compartment C_1 (k_{01}), with elimination (K_{10}) from C_1 , with inter-compartmental distribution rate constants to C_6 (k_{16} , k_{61}). Compartments C_3 , C_4 , and C_5 were added to represent 3-OH, 4-OH, and MEGX, respectively (**Figure 1b**).

A one-compartment absorption and elimination model was used to describe tp LD application. Lidocaine was absorbed from the cutaneous compartment (C_0) to central compartment C_1 (k_{01}) and elimination (K_{10}) from C_1 . Compartments C_3 and C_5 were added to represent 3-OH and MEGX, respectively (**Figure 1c**). Concentrations of the 4-OH were inconsistent and all below the LLOQ and could not be incorporate into the tp model.

The inter-compartmental micro-rate constants for the iv and sq administration were converted to macro-constants exponents, alpha (α), beta (β), and gamma (γ), as described (Boston, Greif et al., 1981; Wastney, Patterson et al., 1999; Stefanovski, Moate et al., 2003). The apparent volume of central compartment (V_C), was calculated by the amount of drug in the body at time t, divided by the plasma concentration at time t (Toutain & Bousquet-Melou, 2004).

Various schemes were used for weighting the data ($W(K)$) in the fitting process. The fractional standard deviation (FSD) was in the form of $W(K)=1/(C*QO(K)**2)$ where $QO(K)$ was the kth observed datum and C was the FSD. The standard deviation (SD) weighting scheme was of the form $W(K)=1/C**2$. The FSD weighting process favors the terminal lower concentrations of the decay curve, where the SD favors the larger and intermediate data points. Data from each horse were iterate multiple times; reducing the estimated SD or FSD at each iteration until the sums of squares and the FSD of each compartmental parameter were no longer

minimized and the observed and predicted lines converged. The fitting process (iterations) ceased when the improvement in the sums of squares of the last iteration was < 1% (Wastney, Patterson et al., 1999).

Calculation of Secondary parameters. The A, B, and C macro-constants coefficients (ng/mL) for the IV administration were calculated from the dose, volume of central compartment (V_C), and the relevant compartmental rate constants (Gabrielsson & Weiner, 2006). Absorption and elimination half-lives (h) were calculated as natural log of two divided by the relevant absorption (K_{ab}) and elimination rate constants (K_e). The plasma concentration time-curves of LD and metabolites were converted to moles and the area under the concentration curve (AUC_0^{lst}) was calculated by the linear trapezoid method. Maximum plasma concentration (Cmax), time to reach the maximum plasma concentration (Tmax), and the AUC were derived directly from the plasma concentration time-curves not the fitted curves. Estimated volumes of compartments C_2 and C_6 following iv administration were calculated by the ratios of the inter-departmental rate constants k_{12}/k_{21} and k_{16}/k_{61} times volume V_C . The volume at steady state (V_{ss}) was calculated as:

$$V_{ss} = V_C \left[1 + \frac{k_{12}}{k_{21}} + \frac{k_{16}}{k_{61}} \right]$$

Compartmental clearance (Clc) was calculated as:

$$Clc = K_e * V_C$$

Bioavailability (F) was calculated as:

$$F = \frac{AUC_{ev}}{AUC_{iv}} * \frac{D_{iv}}{D_{ev}}$$

Where, *ev* and *iv* were the extravascular and intravenous administrations. Clearance values for sq and tp administrations were normalized to the dose base on each horses F.

Pharmacokinetic parameter estimates of LD and metabolites were expressed as median and range. Plasma concentrations were expressed as mean and standard deviation

RESULTS

Intravenous (iv) administration. The V_C was 0.59(0.37-0.88) L/kg. Micro-constant estimates and calculated parameters of LD and metabolites are shown in **Table 1**. The plasma concentration-time curve of the parent drug LD and metabolites are shown in **Figure 2** and macro-constant parameters and secondary calculations of LD are shown in **Table 2**.

All 3 metabolites were quantified in all 6 horses within 2 min. There were no significant differences in peak concentrations of 3-OH and MEXG, both occurring between 10 to 20 min. At 16 h, all LD plasma concentrations were at or below the LLOQ. Lidocaine was detected in plasma in only 2 horses at 24 h. Lidocaine metabolite 4-OH was the least abundant of the 3 and not detected beyond 4 h, and 3-OH and MEXG not beyond 10 and 12 h.

Subcutaneous (sq) administration. Micro-constant estimates and calculated parameters of LD and metabolites are shown in **Table 1**. The plasma concentration-time curve of LD and metabolites are shown in **Figure 3**. Lidocaine K_{ab} and $t_{1/2\ ab}$ were 1.46(1.13-2.37) /h and 0.47(0.29-0.61) h, respectively. The 2-compartment elimination macro constants α and β were 4.66 (2.61-10.67) and 0.23 (0.13-0.27) /h and half-lives $t_{1/2\ \alpha}$ and $t_{1/2\ \beta}$ 0.15 (0.06-0.27) and 3.04 (2.53-6.39) h, respectively.

Plasma concentrations of LD and MEXG were seen in all horses at 2 min, and 3-OH and 4-OH at 5 and 10 min; lidocaine and MEXG were still detected at 24 h. The bioavailability of the sq LD, 3-OH, 4-OH, and MEXG, were 87.7±16.7, 94.4±26.8, 73.4±24.9, and 89.5±22.5 %, respectively.

Topical (tp) administration. Micro-constant estimates and calculated parameters of LD and metabolites are shown in **Table 1**. The plasma concentration-time curve of LD and metabolites are shown in **Figure 4**. The K_{ab} following tp administration was 0.08(0.06-0.13) /h and $t_{1/2\ ab}$ 8.49(5.16-11.08) h. The bioavailability of the LD and metabolites 3-OH and MEXG were 10.2±4.8, 14.4±8.4, and 19.7±10.9 %, respectively.

Urine parameters and concentrations. The urinary AUC, 24, 48 and 72 h urinary concentrations and other parameters following the iv, sq, and tp administrations are shown in **Table 3**. In the first 24-h following the administration of iv LD the percentage elimination of LD, 3-OH, 4-OH, and MEXG, were 22.6±18.0, 51.5±13.9, 2.8±1.0, and 23.1±17.9 %, respectively. The concentration of LD in urine following iv LD was initially high, followed by a rapid decline in the % of total LD excreted, with a concurrent rapid increase in the metabolites 3-OH and MEXG (**Figure 5a**).

A similar, but less pronounced pattern was seen following the sq administration (**Figure 5b**). Following the administration of sq LD, the percentage elimination of LD, 3-OH, 4-OH, and MEXG, were 13.6±14.7, 59.1±15.5, 2.5±1.1, and 24.6±8.7 %, respectively.

The tp urinary excretion pattern reflects slow absorption and elimination and percentage elimination of LD, 3-OH, 4-OH, and MEXG, were 12.7 ± 11.3 , 61.2 ± 11.5 , 2.6 ± 0.90 , and 23.4 ± 5.4 %, respectively (**Figure 5c**).

DICUSSION

The analysis of LD and the 3 metabolites in this study was not a complete analysis of the metabolism of LD as a number of secondary and tertiary metabolites have been described, but not analyzed in this study (Harkins, Mundy et al., 1998). A more complete model has been suggested which requires analysis of primary, secondary and tertiary metabolites (Pang, 1985). Three hydroxylidocaine, 4-OH, and MEXG were selected as they are primary metabolites of the parent compound, as no intermediates have been described (**Figure 6**). Lidocaine and MEXG were detected primarily in the free-form state whereas 3-OH and 4-OH were only present in the conjugated state; detected and quantified only following enzyme hydrolysis. In humans, 4-OH was only present as a glucuronide and MEXG as a free and conjugated form. Glycyl-2,6-xylidide (GX) commonly detected in LD studies is a metabolite of MEXG, which was found in free form (Tam, Ke et al., 1990). Glycyl-2,6-xylidide (GX) has been quantified in LD administrative studies in horses, it was not used in this study as it is a secondary metabolite of MEXG.

Intravenous administration

Median V_C for LD was 0.59 L/kg, a volume of distribution which was cross checked by using model-free method and nonlinear regression methods. The Cl_c of all three metabolites were based on the volume of the central compartment as the metabolites were considered as part of the central pool.

In our study, a 3-compartment model best described the elimination of LD following iv administration. Three-compartment models have been described in earlier studies in humans and more recent studies in cats (De Jong, Heavner et al., 1972; Tucker & Mather, 1975; Nation, Triggs et al., 1977; Thomasy, Pypendop et al., 2005). A prior study in 3 horses using a 2-compartmental analysis, Cl was 52 ± 11.7 and 43.7 ± 8.5 ml/min/kg in control and fasted horses, with $t_{1/2\beta}$ of 0.61 h; a two-compartmental analysis was used based on the limited time (60 min) for which LD was quantified (Engelking, Blyden et al., 1987). Our study reported a Cl_c of ~ 38 ml/min/kg following iv administration of LD. Figures 2 and 3 show concentrations below the LLOQ. Concentrations below the LLOQ for the iv administration were at 16, 20, and 24 h

time points and at these time points 6 were below the LLOQ. Exclusion of data points below LLOQ, biases the best fit curve slightly upward and the elimination rate will be slightly under estimated (Beal, 2001; Clausen, Tabanera et al., 2005; Krishnamoorthy, Mallick et al., 2009). The LLOQ was added to demonstrate the relationship of the samples below the LLOQ to the predicted line. Lidocaine in the equine has been used as a constant infusion. In a 96 h LD infusion study, LD, MEXG, and glycinexylidide (GX) were quantified and at the terminis of the infusion period, the GX plasma concentration was higher than that of LD and MEXG (De Solis & McKenzie, 2007; Dickey, McKenzie et al., 2008). This was not unexpected as MEXG is further metabolized to both GX and 3-hydroxymonoethylglycinexylidine (Harkins, Mundy et al., 1998). This observation was consistant with our study in that MEXG concentrations were higher than LD in both plasma and urine, but lower than 3-OH.

In horse studies cited, the elimination of LD was not influenced by prolonged infusion and following cessation there was a rapid decline in the plasma concentration. This is contrary to studies in man and dogs. In dogs, daily iv administration did not alter the kinetics of LD elimination (Ngo, Tam et al., 1997), but 24 h continuous infusion as compared to 90 min infusion did reduce clearance, due to an impairment of hepatic extraction (LeLorier, Moisan et al., 1977). Long-term intravenous infusion of LD (24 – 36 h) in human subjects, clearance of LD declined during the continuous infusion (Prescott, Adjepon-Yamoah et al., 1976; Nation, Triggs et al., 1977; Bauer, Brown et al., 1982; Thomson, Elliott et al., 1987; Thomson, Kelman et al., 1987).

Hepatic flow in the horse at rest was reported at 23.8ml/min/kg (Dyke, Hubbell et al., 1998), which was lower than our reported Cl of ~38.3 ml/min/kg. In a comparative IV study, the Cl of lidocaine in horses was 2 times greater (~52.0 vs. ~20.6 ml/min/kg) than in humans. The higher clearance of LD in horses suggests extrahepatic metabolism (Engelking, Lofstedt et al., 1987). Extra hepatic drug metabolism has been described for LD and major metabolites MEXG and 3-OH when comparing an-hepatic and controls rabbits (Nyberg, Mann et al., 1996), studies have also suggested that LD is metabolized by the rat lung (Tanaka, Oda et al., 1994), and extrahepatic production of MEGX from LD was measured in a an-hepatic female while awaiting liver transplantation (Sallie, Tredger et al., 1992).

Subcutaneous administration

Following its sq administration the $t_{1/2\text{ab}}$ of LD was 0.47 h (28 min) and LD and the three metabolites were quantified in plasma within 2 to 10 min, with Tmax between 20 and 60 min (Table 1). This can also be noted in **Figure 3** where the LD metabolites appear very quickly following the sq administration. Rapid absorption following sq infiltration of LD was described in earlier studies (Courtot, 1979; Kristinsson, Thordarson et al., 1996).

This study describes the absorption of LD from a site over the carpal bone an area of moderate subcutaneous tissue and vascularity. Following the administration of equal amounts of drug in various locations, it has been shown that the vascularity of the site of injection causes a major difference in the plasma concentration; an obvious comparison was an intercostal vs. an epidural block (Scott, Jebson et al., 1972). The specific drug used will also influence the concentrations reached, LD was absorbed more rapidly than prilocaine (Braid & Scott, 1965). Fractional absorption (bioavailability) of SQ LD in our study was $87.7 \pm 16.7\%$. It must be pointed out that the data presented was sq administration in carpal area; plasma or urine concentrations could be different if injected into areas of lesser or higher blood flow, such as epidural space, or the metatarsal area with a more direct venous flow to the portal circulation.

Topical administration

The median $t_{1/2\text{ab}}$ and $t_{1/2\text{e}}$ into and from the central compartment for tp LD administration was 8.64 and 3.85 h, respectively. This was a flip-flop model where the elimination from the central compartment was determined by slow absorption from the site of tp application. In rat studies measurements during a 16 h period, LD concentrations in the dermis, subcutaneous, fascia, muscle tissues remained high compared to plasma indicating the retention in the sq tissues and slow removal. There was also a 25 min lag period before LD penetrated the epidermis and appeared in the receptor compartment (Singh & Roberts, 1994). Compared to the rapid absorption of LD and appearance of metabolites following sq administration; following tp administration, plasma concentrations of LD and 3-OH were quantified at 10 min and 45 min for MEXG. Throughout the 72-h collection period plasma concentrations remained below 2 ng/ml. The fractional absorption for tp application was $10.2 \pm 4.8\%$ compared to 87.7 ± 16.7 for sq LD.

It is difficult to compare various topical administration studies due to the variability in the site, duration, composition, and concentration of the medication. A study in humans tested the absorption of 4 over-the-counter and compounded topical preparations. Thirty grams of the test preparations were applied over a larger surface area (face and neck) for 60 min. Variability in

plasma concentrations was based on the formulation, LD concentrations (2.5, 4 and 6%) and subject variation. The peak serum concentrations ranged from ~25 to ~425 ng/ml (Oni, Brown et al., 2012). Topical administration studies in other species showed much higher concentration than in our study suggesting slower penetration through equine skin. This may be a function of species variability, where marked difference have been reported in skin layers, follicle density and other physiological characteristics which account for the differences in transdermal penetration (Mills & Cross, 2006). Regional differences have also been reported in the same species, including the horse where significantly higher maximum flux was measured when hydrocortisone was applied to skin from the leg, compared to thorax and groin (Mills, Magnusson et al., 2004; Mills, Magnusson et al., 2005; Mills & Cross, 2006).

Lidocaine was not detected in the horse following the placement of two patches of 5% lidocaine above the medial aspect of the carpus on both fore limbs. The quantification was by ELISA (Neogen Corporation USA) with a reported sensitivity of 1 ng/ml (Bidwell, Wilson et al., 2007).

In comparison, a lidocaine patch in cats produced steady-state LD and MEGX plasma concentrations of ~0.083 and ~0.012 µg/ml. Overall bioavailability of transdermal lidocaine was ~6.3 % and skin concentrations were much higher than plasma at (211 µg/g) (Ko, Maxwell et al., 2008). Lidocaine patches have been used safely in humans and dogs (Gammaitoni, Alvarez et al., 2003; Ko, 2007). These differences seen in this equine study may simply be related to the clinical technique used to prepare skin, the formulation used which can significantly affect the rate and extent of penetration of a topically applied drug (Mills & Cross, 2006).

Our study was in the intact skin, but creams containing lidocaine/antibiotics are commonly used in the horse for skin lacerations and abrasions, therefore the more common applications are in traumatized surfaces. Chronic leg ulcers were covered for 24 h with a lidocaine-prilocaine topical cream (EMLA) (25 mg/g). Plasma concentrations in these human patients ranged from 185-705 ng/ml between 2-4 h (Stymne & Lillieborg, 2001). In a comparative study application onto damaged anorectal mucosa (45 mg), median C_{max} and T_{max} were 73.6 ng/ml and 40 min, compared to normal mucosa where none was detected (Perrotti, Grumetto et al., 2006; Perrotti, Dominici et al., 2009). Two groups of mice were compared, one with intact vs lacerated skin. The T_{max} was similar, but C_{max} ranged from 165.7 to 909.2 ng/ml in the intact vs the lacerated skin (Al-Musawi, Matar et al., 2012).

Urinary concentrations

The most abundant metabolite in urine in this study was 3-OH and in all 3 administrations represented ~57 % of the total eliminated in urine. Lidocaine represented ~16 % of the LD administered, where 4-OH and MEXG represented ~3 and ~24 % of the total. It should be noted that the plasma concentrations of 3-OH and MEXG were superimposed, but the urinary concentration of 3-OH were higher than MEXG. MEXG is metabolized to GX and 4-hydroxymonoethylglycinexylidine which were not measured, which would account for the lower urinary concentration.

In summary, this study confirms the one published IV bolus administration in the horse that the clearance of LD was higher than the reported hepatic blood flow which also suggested significant metabolism at extrahepatic sites. Following sq administration LD was quantified in plasma within two min with comparable clearances from the central compartment for the iv and sq administrations. Following tp application plasma concentrations of LD and metabolites were all below 2 ng/ml and the fractional absorption were low compared to studies in other species. Despite the low plasma concentration LD and metabolites were quantified in urine at 72 hours. In this study only one commercial preparation was used and applied in only one location but compared to studies in other species the absorption was slow and plasma concentrations low.

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Table 1. Pharmacokinetic parameter estimates (median and range) of the compartmental analysis of lidocaine (LD) and metabolites, 3-hydroxylidocaine (3-OH), 4-hydroxylidocaine (4-OH), and monoethylglycinexylidide (MEGX), following a single iv, sq, and tp administrations in 6 horses.

Intravenous Lidocaine (100 mg)				
Parameter	LD	3-OH	4-OH	MEGX
K_e (/h)	4.12 (2.62-6.23)	1.25 (1.10-2.15)	1.79 (1.22-2.39)	1.69(1.03-1.99)
$t_{1/2e}$ (h)	0.17(0.11-0.26)	0.55(0.32-0.63)	0.39(0.29-0.57)	0.41(0.35-0.67)
Cl_C (L/h/kg)	2.30(2.11-2.81)	0.82(0.45-1.44)	1.06(0.61-2.11)	1.10(0.38-1.57)
AUC_0^{lst} (ng•h/mL)*	56.9(51.3-67.6)	20.9 (9.7-24.7)	0.83 (0.29-0.10) ^c	14.7 (12.4-24.5)
T_{max} (min)	2	10(10-20)	20(10-20)	10(10-20)
C_{max} (ng/ml)	277.4(231.1-330.5)	17.6(6.4-20.5)	0.78(0.34-1.04)	9.4(8.5-18.7)
Subcutaneous Lidocaine (200 mg)				
K_e (/h)	3.91 (1.48-9.25)	1.00 (0.78-1.08)	1.76 (0.96-2.11)	1.13(0.69-1.33)
$t_{1/2e}$ (h)	0.18(0.07-0.45)	0.69(0.64-0.88)	0.39(0.33-0.72)	0.61(0.52-0.99)
Cl_{C*F} (L/h/kg)	2.48(2.23-3.82)	0.71(0.32-1.78)	1.02(0.40-1.67)	0.73(0.42-1.35)
AUC_0^{lst} (ng•h/mL)*	104.5(72.4-163.5)	28.9 (20.2-52.4)	1.3 (0.86-2.1)	27.3 (20.3-38.4)
T_{max} (min)	20(10-20)	45(30-60)	50(45-60)	45(30-60)
C_{max} (ng/ml)	94.6(66.7-117.2)	12.2(8.7-19.9)	0.54(0.36-0.88)	10.4(8.3-15.1)
Topical Lidocaine (400 mg)				
K_e (/h)	0.24 (0.10-0.81)	0.41 (0.08-0.93)		0.38(0.26-1.14)
$t_{1/2e}$ (h)	2.91(0.84-6.68)	1.67(0.75-8.46)		1.80(0.61-2.70)
Cl_{C*F} (L/h/kg)	1.25(0.30-2.06)	2.39(0.36-3.86)		3.44(1.35-8.09)
AUC_0^{lst} (ng•h/mL)*	23.3(10.9-41.8)	12.5(4.4-22.4)		12.2(6.1-19.7) ^b
T_{max} (h)	4.0(2.0-12.0)	4.0(3.0-8.0)		5.0(3.0-8.0)
C_{max} (ng/ml)	2.17(1.19-2.84)	0.89(0.10-1.70)		0.93(0.48-1.27)

K_e = elimination rate constants; $t_{1/2e}$ elimination half-life; Cl_C = compartmental clearance; AUC_0^{lst} = area under the concentration-time curve, 0 to last hour*; T_{max} =time to maximum concentration; C_{max} maximum concentration; Cl_{C*F} =fractional clearance

*last hour represents 4 to 24 h, depending on the route of administration, LD or metabolite.

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Table 2. Pharmacokinetic macro-constant parameter estimates (median and range) of lidocaine (LD) following a single iv administration of 100 mg in 6 horses.

Parameter	LD IV
A (ng/mL)	373.1(212.6-472.3)
α (h ⁻¹)	8.64(5.28-10.38)
$t_{1/2\alpha}$	0.08(0.07-0.13)
B (ng/mL)	44.2(15.1-6.14)
β (h ⁻¹)	1.28(0.55-4.60)
$t_{1/2\beta}$ (h)	0.57(0.15-1.25)
C (ng/mL)	2.90(1.83-3.83)
γ (h ⁻¹)	0.17(0.09-1.32)
$t_{1/2\gamma}$ (h)	4.11(0.52-7.36)
V _C (L/kg)	0.59(0.37-0.88)
V ₂ (L/kg)	1.31(0.87-2.41)
V ₃ (L/kg)	0.93(0.37-1.73)
V _{ss} (L/kg)	3.24(1.61-4.95)

A, B, C = coefficients; α , β , γ = exponents; $t_{1/2\alpha}$, $t_{1/2\beta}$, $t_{1/2\gamma}$ = half-lives; V_C = volume of central compartment;

V₂, V₃ = volume of compartments 2, 3; V_{ss} = volume of distribution at steady state.

Table 3. Urinary values (median and range) of lidocaine (LD) and metabolites, 3-hydroxylidocaine (3-OH), 4-hydroxylidocaine (4-OH), and monoethylglycinexylidide (MEGX), following a single iv, sq, and tp administration in 6 horses.

Intravenous Lidocaine (100mg)				
Parameter	LD	3-OH	4-OH	MEXG
AUC_0^{24} ($\mu\text{g}\cdot\text{h/mL}$)	3.1(0.54-9.7)	12.0(6.5-29.3)	0.61(0.50-1.4)	5.7(3.6-11.7)
$T_{\max}$ (h)	2(0.5-4)	2(1-4)	2(1-4)	2.0(1-4)
$C_{\max}$ (ng/ml)	1702.0(52.4-8053.1)	2898.9(1694.2-6261.7)	203.1(155.5-865.5)	1336.0(731.9-6373.1)
C_{24} (ng/ml)	6.2(1.0-31.3)	16.6(1.1-52.4)	0.73(0.03-3.2)	3.3(0.6-21.3)
C_{48} (ng/ml)	1.6(0.77-9.8)	5.0(1.5-7.9)	0.20(0.05-0.34)	0.47(0.03-3.5)
Subcutaneous Lidocaine (200)				
AUC_0^{24} ($\mu\text{g}\cdot\text{h/mL}$)	2.8(2.7-18.8)	25.4(12.4-33.7)	1.3(0.46-1.4)	6.4(3.1-19.2)
$T_{\max}$ (h)	2(2-6)	2(1-2)	2(1-2)	2(1-2)
$C_{\max}$ (ng/ml)	955.2(423.4-3069.7)	6856.1(5064.2-9089.5)	253.8(167.2-407.9) ^b	1511.1(699.1-4509.1) ^b
C_{24} (ng/ml)	5.4(3.4-29.9)	51.4(23.6-67.4)	1.9(0.68-2.4) ^a	11.0(5.5-20.9) ^a
C_{48} (ng/ml)	1.2(0.07-4.1)	5.4(2.8-10.3)	0.09(0.08-0.48)	1.2(0.29-1.9)
Topical Lidocaine (400 mg)				
AUC_0^{24} ($\mu\text{g}\cdot\text{h/mL}$)	2.3(0.69-6.3)	11.6(4.1-13.5)	0.38(0.23-0.45)	3.9(2.0-5.7)
$T_{\max}$ (h)	10.0(8-16)	9(8-16)	8(8-16)	8(6-16)
$C_{\max}$ (ng/ml)	197.2(79.3-649.6)	552.3(234.7-1059.5)	27.3(15.2-37.7)	169.1(105.5-328.2)
C_{24} (ng/ml)	55.5(9.5-100.3)	265.7(122.9-420.5)	12.2(2.6-17.2)	58.1(46.9-165.7)
C_{48} (ng/ml)	2.8(0.08-37.4)	10.2(4.8-18.1)	0.31(0.18-0.91)	2.2(1.4-3.6)
C_{72} (ng/ml)	2.2(0.62-4.4)	4.0(1.4-10.7)	Not detected	1.2(0.15-1.6)

AUC_0^{24} = of area under the curve 0 to 24 h; $T_{\max}$ = time to maximum concentration; $C_{\max}$ = maximum concentration; C_{24} , C_{48} , C_{72} = urinary concentrations at 24, 48, 72 hr

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