Identification of lidocaine and its metabolites in post-adminstration equine urine by ELISA and MS/MS[1,2]

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Lidocaine is a local anesthetic drug that is widely used in equine medicine. It has the advantage of giving good local anesthesia and a longer duration of action than procaine. Although approved for use in horses in training by the American Association of Equine Practitioners (AAEP), lidocaine is also an Association of Racing Commissioners International (ARCI) Class 2 drug and its detection in forensic samples can result in significant penalties.

Lidocaine was observed as a monoprotonated ion at m/z 235 by ESI^+ MS/MS (electrospray ionization-positive ion mode) analysis. The base peak ion at m/z 86, representing the postulated methylenediethylamino fragment $[CH_2N(CH_2CH_3)_2]^+$, was characteristic of lidocaine and 3-hydroxylidocaine in both ESI^+ and EI (electron impact-positive ion mode) mass spectrometry. In addition, we identified an ion at m/z 427 as the principal parent ion of the ion at m/z 86, consistent with the presence of a protonated analog of 3-hydroxylidocaine-glucuronide.

We also sought to establish post-administration ELISA-based 'detection times' for lidocaine and lidocaine-related compounds in urine following single subcutaneous injections of various doses (10, 40, 400 mg). Our findings suggest relatively long ELISA based 'detection times' for lidocaine following higher doses of this drug.

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INTRODUCTION

Lidocaine is an amide-type local anesthetic drug that is widely used in horses (Soma, 1971). The Association of Racing Commissioners International (ARCI) classifies lidocaine as a Class 2 foreign substance (Tobin, 1995), and its identification in post-race urine samples can result in significant penalties (Tobin, 1981). We, therefore, chose both to investigate the detection and identification of lidocaine and its major urinary metabolites and to further determine the ELISA based 'detection times' for this drug in post-administration urine samples.

Lidocaine is a relatively stable local anesthetic drug (Soma, 1971). Compared to procaine, lidocaine has a faster onset and longer duration of action, at least partly because procaine, an ester, is rapidly hydrolyzed by plasma and liver esterases (Tobin *et al.*, 1977). The primary pharmacological activity of lidocaine and other local anesthetic drugs involves inhibition of the excitation-conduction process in peripheral nerves by preventing the increase in membrane permeability to sodium ions (Butterworth & Strichartz, 1990).

Lidocaine is predominantly metabolized in the liver. Reported metabolites of lidocaine in the horse include 3-hydroxylidocaine, dimethylaniline, 4-hydroxydimethylaniline, monoethylglycinexylidine (MEGX), 3-hydroxy-monoethylglycinexylidine and glycinexylidine (Short *et al.*, 1988).

We have previously (Harkins *et al.*, 1998) determined the highest no-effect dose (HNED) of lidocaine by using an abaxial sesamoid block model (Harkins *et al.*, 1996). Using this model, the HNED for lidocaine was determined to be 4 mg. Following subcutaneous injection of the HNED of lidocaine, the concentration of 3-hydroxylidocaine recovered from urine reached a peak of about 315 ng/mL at 1 h after administration. These data suggest that recovery of less than 315 ng/mL of 3-hydroxy-

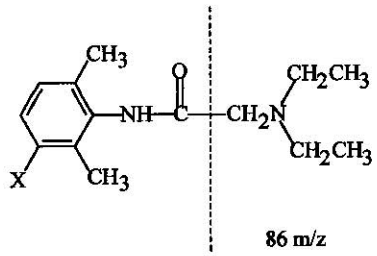


Fig. 1. Schematic diagram illustrating fragmentation of lidocaine-related molecules and the origin of the 86 m/z fragment in mass spectra. For lidocaine X = H (235 m/z); for 3-hydroxylidocaine (251 m/z), X = OH; for the glucuronide of 3-hydroxylidocaine (427 m/z), X = $HOOCC_5H_8O_5$.

lidocaine from a post-race urine sample is unlikely to be associated with a local anesthetic effect of lidocaine.

The objectives of this study were to detect and directly identify the major urinary metabolite of lidocaine found in post-administration urine samples and to determine the relationship between lidocaine dose and apparent urinary 'detection times' for lidocaine after clinical doses of this drug when detection is based on ELISA testing. The hypothesized structure of the major equine urinary metabolite of lidocaine is presented in Fig. 1, along with a listing of the major mass spectral ion fragments of this molecule (Table 1).

MATERIALS AND METHODS

Horses and urine collection

Seven mature thoroughbred mares weighing 413–602 kg were used for this study. All horses were acclimated to their stalls 24 h prior to experimentation. The animals were maintained on grass hay and feed (12% protein), which was a 50:50 mixture of oats and an alfalfa-based protein pellet. Horses were fed twice a day. The animals were vaccinated annually for tetanus and were dewormed quarterly with ivermectin. A routine clinical examination was performed prior to each experiment to assure those animals were healthy and sound. During experimentation, horses were provided water and hay *ad libitum*. Horses were randomly injected subcutaneously with a single dose (4, 40 or 400 mg) of 2% lidocaine HCl (Elkins–Sinn, Inc., Cherry Hill, NJ, USA). The site of injection was the lateral volar nerve where it passes lateral (abaxial) to the lateral sesamoid bone. During the first day, complete urine collection was accomplished with a Foley catheter at 0, 1, 2, 4, 6 and 8 h after administration. At 24, 48, 72 and 96 h after administration, a Harris flush tube (24 Fr × 152.4 cm; Seamless, Ocala, FL, USA) was used to collect urine samples. Urine was placed in aliquots, and stored at −20 °C until assayed.

Table 1. Interpretation of principal ions seen as daughters of putative lidocaine-O-glucuronide 427 m/z ion. Results summarized as averages from several runs. Listed in order of decreasing average relative intensity

Ion, m/z	Relative intensity	Interpretation
427	100%	Lidocaine-glucuronide + 1
86	90%	Methylenediethylamino fragment
251	50%	Hydroxylidocaine + 1 obtained by loss of glucuronic acid moiety
202	46%	Internal fragment

General MS/MS methods

Full scan electrospray ionization (ESI) mass spectra were obtained on analytical standards at 10 μg/mL in methanol:1% formic acid (aq), 50:50 by infusion at 0.9 mL/h via a Harvard syringe pump into the electrospray probe of a Micromass Quattro II MS/MS (Micromass, Beverly, MA, USA) operated in positive ion mode. All mass spectra were optimized by combination of 1–2 min of uniformly acquired data, background subtraction and peak smoothing. Both daughter and parent ion analyses were also performed. Complex spectra were converted from continuum data to centroid data.

MS/MS calibration. After first tuning the ESI^+ source with 1:1 acetonitrile: 2 mM ammonium acetate, 0.1% formic acid, the instrument was calibrated with a mixture of polypropylene glycols (PPGs) of average molecular weights 425, 725, 1000 and 2000. These were dissolved in 1:1 acetonitrile: aqueous 2 mM ammonium acetate at 20–50 ng/μL depending on the PPG. Calibration utilized the following monoisotopic ions: 59.04969, 291.2172, 541.3952, 964.7148, 1312.9660 and1835.3427 amu (Note that 59–233 are fragments of the form $(C_3H_6O)_n$, 291–541 are $(PPG + H^+)$, and above 616 are $(PPG + NH_4^+)$). Calibration enabled the accurate assignment of masses within the +1–2 Da window factory adjustment for each mass value.

MS/MS tuning. The mass spectrometer was tuned by direct injection of 10 ng/μL lidocaine in methanol:1% formic acid (aq), 50:50. The peak shape and intensity of the monoprotonated lidocaine ion at m/z 235 were optimized by adjustment of capillary, HV lens, cone voltage, skimmer lens and RF lens settings. Skimmer lens offset was left at 5v. Collision gas (argon) and collision energy were adjusted for collisionally induced dissociation (CID) in the central hexapole by optimizing settings for production of the daughter ion at m/z 86 from the ion at m/z 235 in MS2. Generally the collision gas was set to 3×10^{-3} mbar. Increasing the photomultiplier setting 100–150 V above the normal 650 V was found to increase our sensitivity sufficiently. In general, the source cone voltage was set at +25 V, the collision energy was set between −50 and −70 V, the capillary of the ESI probe was set at +3.60 kV and the HV lens was set at +0.7 kV. Source temperature was set at 120 °C.

Sample preparation. Lidocaine base was from Sigma Chemicals (St Louis, MO, USA), 3-hydroxylidocaine was prepared as previ-

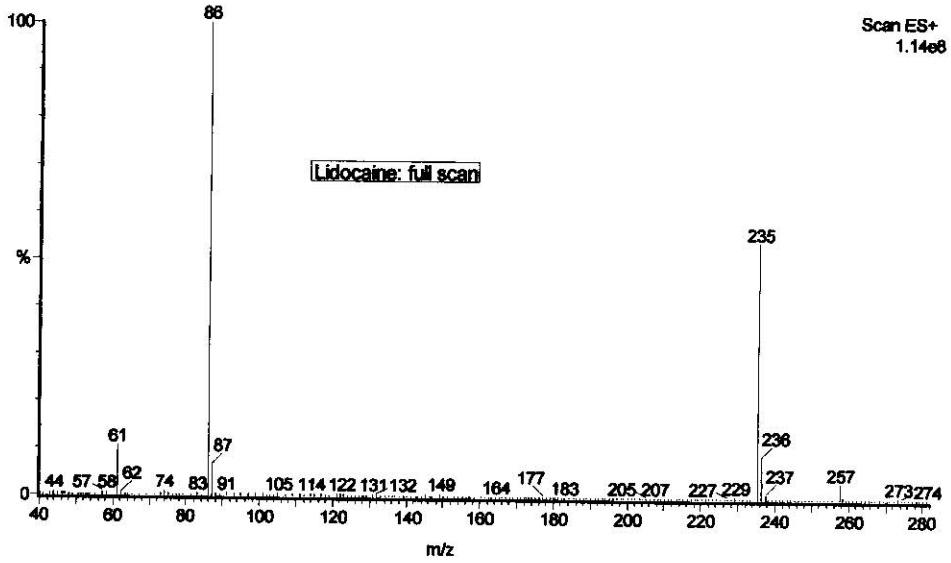


Fig. 2. Full scan electrospray mass spectrum of lidocaine at 10 μg/mL in methanol:1% formic acid (aq), 50:50 infused at 0.9 mL/h via Harvard syringe pump into the electrospray probe of the Quattro II MS/MS set in positive ion mode.

ously described by Harkins *et al.* (1998). Urine samples from horses treated 2 h previously with a 400 mg subcutaneous dose of lidocaine were passed through a 3000 m.w. cutoff Centricon-3 filter (Amicon, Inc., Beverly, MA, USA; a division of Millipore) to remove high molecular weight materials. Specifically, 900 μL urine was centrifuged 60–90 min at $2000 \times g$ in a swinging bucket rotor centrifuge (Type AH-4, Beckman AccuSpinFR, Beckman, Palo Alto, CA, USA). The filtrate was diluted 1:10 with methanol:1% formic acid (aq), 50:50. The mixture was infused at 0.9 mL/h via a Harvard syringe pump equipped with a 500 μL Hamilton gas-tight syringe. Infusion was direct into the electrospray probe of the Quattro II MS/MS set in positive mode.

For glucuronide hydrolysis experiments, urine samples collected from horses for 2 h after administration of 400 mg of lidocaine subcutaneously were treated for 3 h at 65 °C with beta-glucuronidase (1000 units of Sigma Type L-II per mL of urine brought to 0.25 M sodium acetate, pH 5). Resultant mixtures were centrifugally filtered and diluted as above.

ELISA (enzyme linked immunosorbent assay) analysis

General ELISA methods. The one-step ELISA tests were performed as previously described (Voller *et al.*, 1976). All assays were performed at room temperature. Briefly, anti-lidocaine antibodies were linked to flat bottom wells. Similarly, the lidocaine hapten was linked to horseradish peroxidase (HRP) to create a covalently linked lidocaine–HRP complex. The lidocaine test is commercially available from Neogen Corporation of Lexington, KY, USA. The optical density (OD) of each well was read at a wavelength of 650 nm with an automated microplate reader (EL 310, Microplate Autoreader, Bio-Tech. Inc., Winooski, VT 05404, USA). Samples exceeding the OD of the highest standard were diluted appropriately with assay buffer and rerun.

RESULTS

MS/MS characteristics of authentic standards

Lidocaine and 3-OH-lidocaine. Fig. 2 presents a full scan electrospray positive mode (ESI^+) mass spectrum of authentic lidocaine. Lidocaine (10 μg/mL in methanol: 1% formic acid (aq) 50:50) was infused at 0.9 mL/h with Harvard syringe pump into the electrospray probe of the Quattro II MS/MS set in positive ion mode. Lidocaine (234 m.w.) was observed as a monoprotonated ion at m/z 235. The base peak ion at m/z 86, representing the methylenediethylamino $[CH_2N(CH_2CH_3)_2]^+$ fragment, is the characteristic ion of lidocaine and related compounds (e.g. hydroxylidocaine and lidocaine glucuronide) containing this moiety both in ESI^+ and EI mass spectrometric determinations, as shown in Fig. 1. Other work showed that the daughter ion spectrum of protonated lidocaine and the parent ion spectrum of the ion at m/z 86 confirm the relationship between these ions (data not shown).

Authentic 3-hydroxylidocaine (250 m.w.) was observed as a monoprotonated ion at m/z 251 by ESI-MS/MS, with the base peak ion at m/z 86 confirming the relationship to lidocaine (Fig. 3). Both the daughter ion spectrum of the protonated 3-hydroxylidocaine and the parent ion spectrum of the ion at m/z 86 (data not shown) confirm the relationship between the 3-hydroxylidocaine and methylenediethylamino ions.

MS/MS analyses of post-administration urine samples

MS/MS identification of 3-hydroxylidocaine glucuronide. Two hour post-administration (400 mg lidocaine) urine samples were filtered, diluted 1:10 with methanol: 1% formic acid (aq), 50:50 and infused into the electrospray probe of the Quattro II MS/MS set in positive mode. In this sample, the diagnostic ion at m/z 86 is readily identified by full scan MS analysis along with an ion at m/z 427, which is consistent with the protonated analog of a hydroxyphenyl glucuronide of lidocaine; Fig. 4 shows a

region of the full scan spectrum displaying the ion at m/z 427. Fig. 5 shows that this ion is the principal parent of the ion at m/z 86 by parent ion MS/MS spectrometry. Additionally, Fig. 5 shows the presence of an ion at m/z 251 consistent with hydroxylated lidocaine, and an ion at m/z 114 which may represent hydrolytic cleavage of the amide linkage of lidocaine or its metabolites to yield the fragment $[COCH_2N(CH_2CH_3)_2]^+$ which is also likely to be capable of fragmentation to the ion at m/z 86.

Other experiments, showing the daughter ion spectrum of the ion at m/z 427 (Fig. 6) from a lidocaine administration urine sample, further confirm the link between the ions at m/z 86 and 427. In this case, the ion at m/z 86 again represents fragmentation to $[CH_2N(CH_2CH_3)_2]^+$], and the ion at m/z 251 represents breakage of the O-glucuronide bond to release protonated hydroxylidocaine. The interpretation of principal ions seen as daughters of the putative lidocaine-O-glucuronide ion at m/z 427 is in Table 1, based on data summarized as averages from several runs.

Effect of beta-glucuronidase hydrolysis on urinary 3-OH-lidocaine glucuronide. To independently establish that the ion at m/z 427 is due to the glucuronide conjugate of 3-OH-lidocaine, we subjected this material to beta-glucuronidase hydrolysis. Fig. 7 shows the parent ion spectrum of the ion at m/z 86 of a urine sample from a lidocaine-treated horse following beta-glucuronidase treatment for 3 h at 65 °C. The results (Fig. 7) indicate that both the ions at m/z 251 and 114 are the primary parents of the ion at m/z 86. In the same context, disappearance of the parent ion at m/z 427 confirms its identity as both a glucuronide candidate and a relative of lidocaine, most simply as the hydroxyphenyl lidocaine-glucuronide.

In other work we have shown that the daughter ion spectrum of the ion at m/z 251 following beta-glucuronidase treatment is essentially identical to that shown in Fig. 3, confirming the presence of a hydroxylidocaine. Additionally, examination of daughter ions at m/z 427.5 following beta-glucuronidase treatment resulted in lack of appearance of the ion

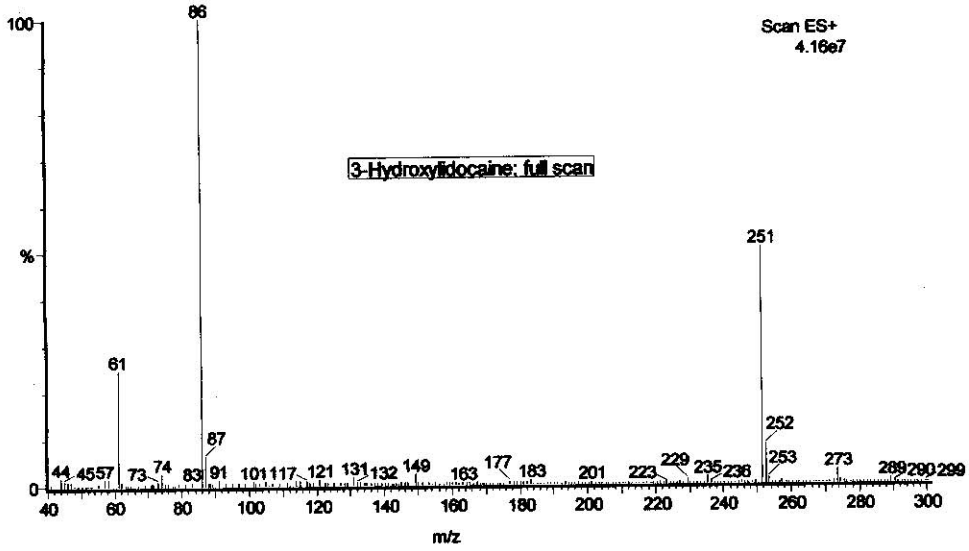


Fig. 3. Full scan electrospray MS of 3-hydroxylidocaine introduced at 10 μg/mL as with lidocaine in Fig. 2. Conditions as in Fig. 2. 3-Hydroxylidocaine (250 m.w.) is observed as a 251 m/z protonated ion; the 273 m/z ion represents complexation with trace background sodium (23 m.w.); 289 possibly the same with trace background potassium (m.w. 39). Note recurrence of the 86 m/z base peak, representative of the methylenediethylamino $[CH_2N(CH_2CH_3)_2]^+$ fragment characteristic of lidocaine relatives.

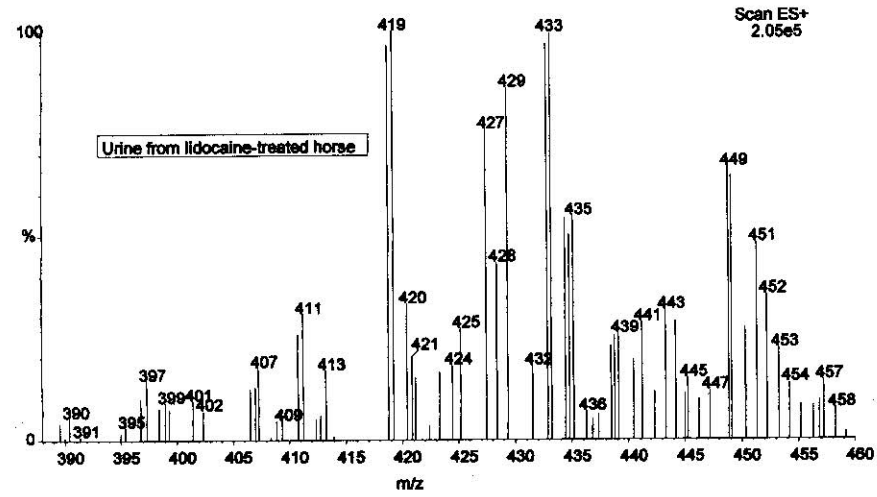


Fig. 4. Full scan electrospray MS of urine from horse # 695, treated 2 h previously with a 400 mg subcutaneous dose of lidocaine. The urine was passed through a 3000 m.w. cutoff Centricon-3 filter (Amicon, Inc.) to remove high molecular weight materials, and diluted 1:10 with methanol:1% formic acid (aq), 50:50. The mixture was infused at 0.9 mL/h via a Harvard syringe pump into the electrospray probe of the Quattro II MS/MS set in positive mode. Conditions for acquisition were as in Fig. 2. The scan region from approximately 388 to 460 m/z has been expanded to demonstrate the presence of the 427 m/z ion which is the parent ion of the diagnostic 86 m/z ion.

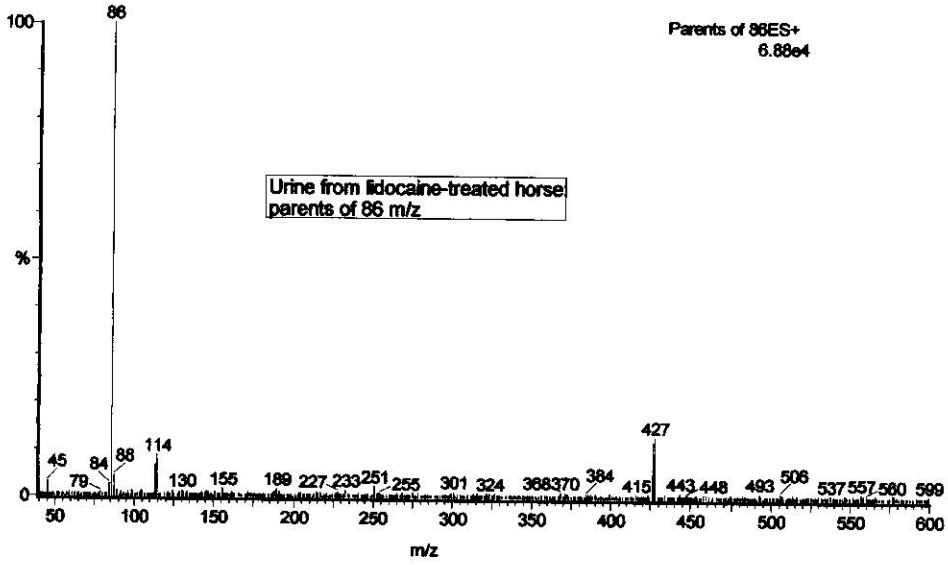


Fig. 5. Parent ion spectometry of 86 m/z ion of urine from horse # 695, treated 2 h previously with 400 mg lidocaine. Parent ion spectrum examining the possible presence of parents of the 86 m/z ion, the characteristic fragment expected from lidocaine metabolites with an unmetabolized 2-(diethylamino)acetamido structure. Note the identification of the 427 m/z ion as a principal precursor to 86 m/z; this matches the predicted molecular weight of a protonated analog of a glucuronide for a hydroxyphenyl metabolite of lidocaine. In this context, the 251 m/z ion represents lidocaine hydroxylated presumably in the phenyl 3- or 4-positions. Based on the areas of the acquired peaks alone, the peak identified as hydroxylidocaine (251 m/z, area 1775) is present to the extent of 9.9% of that representing the lidocaine glucuronide (427 m/z, area 17978). As it could also relate to lidocaine structure, the 114 m/z peak may represent hydrolytic cleavage of the amide linkage of lidocaine or its metabolites to release the $[O{=}CCH_2N(CH_2CH_3)_2]^+$ fragment presumably also capable of fragmentation to 86 m/z. Conditions for acquisition were as in Fig. 2.

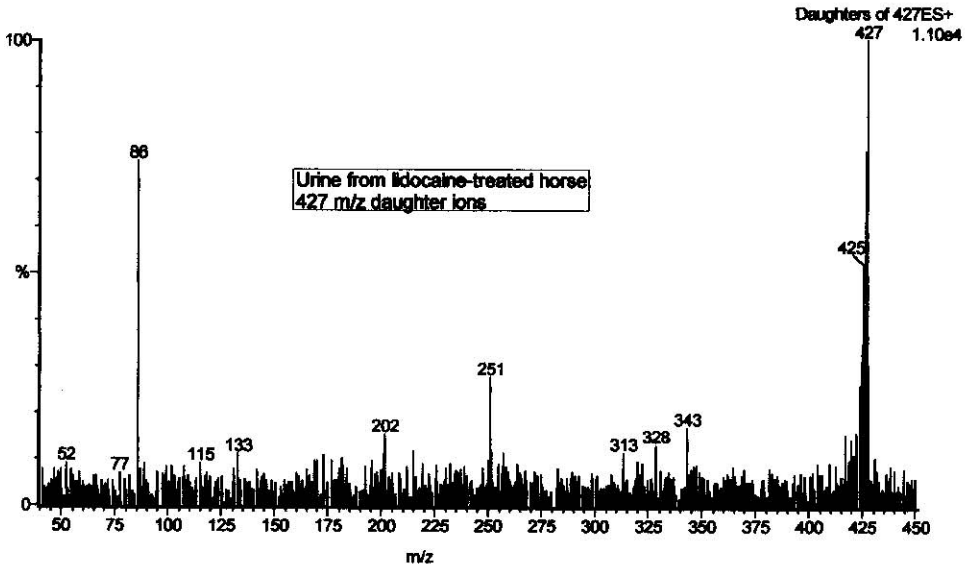


Fig. 6. Daughter ion spectrum examining products released from the 427 m/z ion of urine from horse # 695, treated 2 h previously with 400 mg lidocaine. The 86 m/z ion represents fragmentation to release the $[CH_2N(CH_2CH_3)_2]^+$ fragment, whereas 251 m/z represents breakage of the O-glucuronide bond to release protonated hydroxylidocaine. Conditions as in Fig. 2.

at m/z 86, indicating that only background ions unrelated to lidocaine are present in the 427–428 m/z ion peak (data not shown).

ELISA analyses

These data strongly suggest that the principal metabolite of lidocaine seen in post-administration urine samples is the 3-hydroxyglucuronide conjugate of 3-OH-lidocaine. To determine the period post-lidocaine administration, for which these metabolites are likely to be detected by ELISA analysis, we screened post-lidocaine administration samples for detection of apparent lidocaine by ELISA. Lidocaine ELISA tests were performed in urine samples following subcutaneous administration of lidocaine at doses of 10, 40, and 400 mg/site. All standard curves were generated in ELISA buffer, and samples exceeding

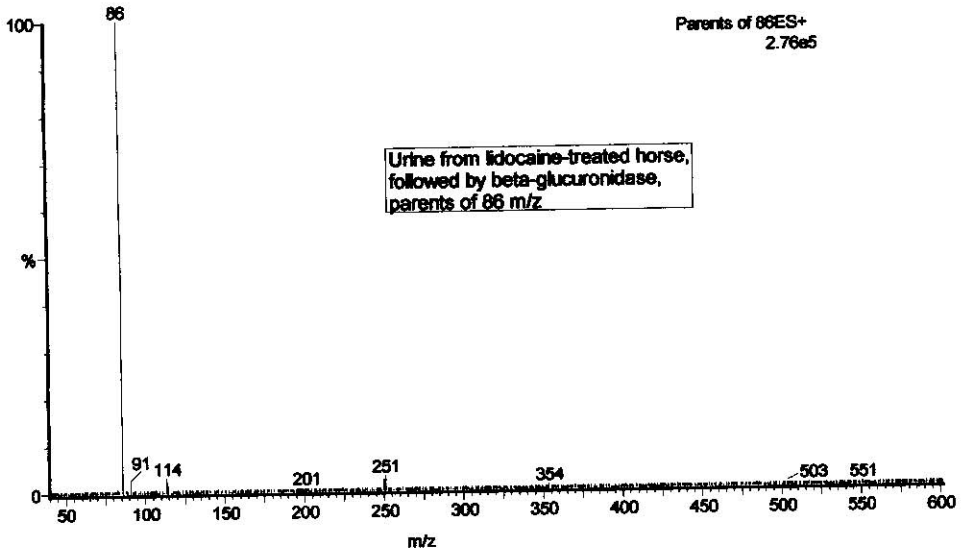


Fig. 7. Parent ion spectrum examining the possible presence of parents of the 86 m/z ion of urine from horse #695, treated 2 h previously with a 400 mg subcutaneous dose of lidocaine and treated for 3 h at 65 °C with beta-glucuronidase. The 427 m/z parent ion has disappeared, confirming its identity as a glucuronide. Conditions for acquisition as in Fig. 2.

the OD of the highest standard were diluted appropriately with assay buffer and reanalyzed. ELISA screening does not specifically identify lidocaine or its metabolites, so all ELISA data are expressed as lidocaine equivalents.

Fig. 8 shows the observed post-administration urinary concentrations of lidocaine equivalents. Peak urinary concentrations were observed at 2 h post-administration and were 1.4 μg/mL (±0.93 SEM), 10.2 μg/mL (±0.54 SEM), and 30.4 μg/mL (±4.166 SEM) at the 10, 40, and 400 mg doses, respectively (Fig. 8). Additionally, there was a linear correlation between peak mean urinary concentrations and doses of lidocaine as shown in Fig. 8 ($r^2 = 0.947$, where r is correlation coefficient).

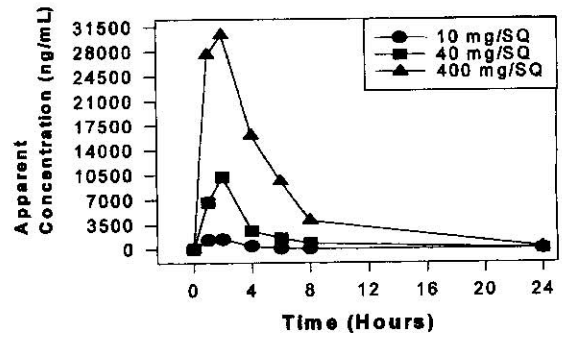
Fig. 9 shows the percent of maximum optical density of the ELISA tests following the incremental subcutaneous doses of lidocaine, with the straight line representing 50% inhibition of the ELISA test.

DISCUSSION

Lidocaine is probably one of the most widely used local anesthetics in horses. As well as being widely used by veterinarians, lidocaine is present in a number of over-the-counter topical antibiotic preparations, to which it has presumably been added for its topical local anesthetic effect. Based on previous work from this group, it is clear that lidocaine administration yields relatively large urinary concentrations of its major metabolites in the horse (Harkins *et al.*, 1998). These observations are consistent with the finding that lidocaine is one of the most commonly reported local anesthetics in racing horses.

Also since lidocaine is listed as a Class 2 substance by the ARCI, its detection in forensic samples can result in significant penalties. On this basis, therefore, information concerning the detection, identification, 'detection times', estimated 'detection times' and 'withdrawal times' for lidocaine and/or lidocaine metabolites in blood or urine samples are very important for the proper use and regulation of this drug by equine forensic scientists.

LC-MS/MS instrumentation enabled us to examine directly the major post-administration urinary metabolite of lidocaine, both before and after beta-glucuronidase treatment. We first



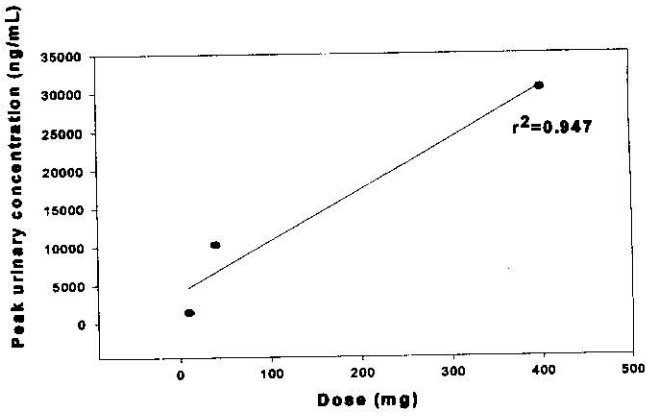


Fig. 8. Mean urinary concentrations of lidocaine equivalents (24 h) following subcutaneous administration of 10, 40, 400 mg of lidocaine. Comparison of peak mean urinary concentrations and doses of lidocaine ($r^2 = 0.947$).

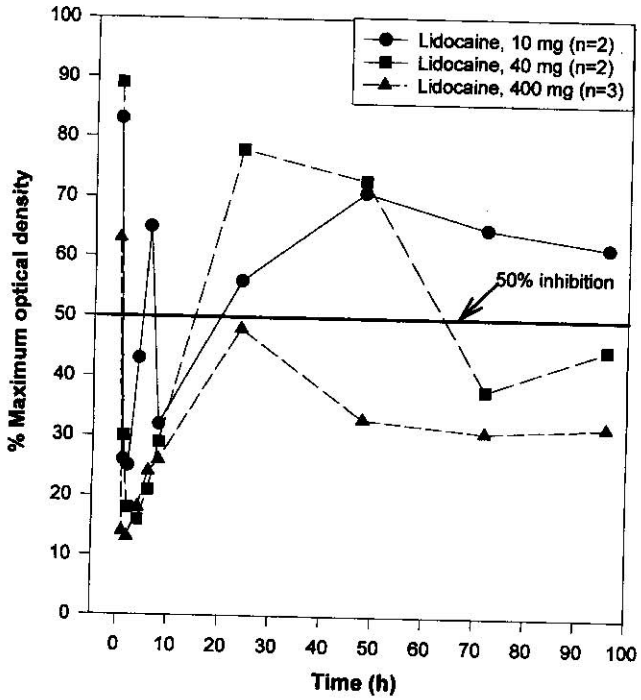


Fig. 9. Percentage of maximum OD of ELISA tests of urine from horses treated with incremental doses of lidocaine. The horizontal line represents 50% of the maximum OD.

established that the base peak ion at m/z 86, attributed to the methylenediethylamino fragment $[CH_2N(CH_2CH_3)_2]^+$, was characteristic of lidocaine and 3-hydroxylidocaine in ESI$^+$ MS, as previously known for EI MS (data not shown). Both lidocaine (234 m.w.) and 3-hydroxylidocaine (250 m.w.) were observed as monoprotonated ions at m/z 235 and 251, respectively. Also, the relationships between the base peak ions and parent ions were confirmed by both daughter ion and parent ion spectrometry (spectra not shown).

We next established that the monoprotonated analog of 3-hydroxylidocaine-glucuronide (427 m/z) was the parent of the 86 m/z ion seen in full ESI$^+$ MS scans of unhydrolyzed urine. This relationship between 427 m/z and 86 m/z was confirmed by daughter ion spectrometry of the 427 m/z ion. The 114 m/z peak presumably represents hydrolytic cleavage of the amide linkage of lidocaine or its metabolites, releasing the $[COCH_2N(CH_2CH_3)_2]^+$ ion which is likely also capable of fragmentation to ion at m/z 86.

We next examined the effect of beta-glucuronidase hydrolysis on the m/z 427 peak putatively associated with presence of 3-hydroxylidocaine glucuronide in post-administration urine samples. Beta-glucuronidase hydrolysis resulted in disappearance of the parent ion at m/z 427 confirming its identity as a glucuronide. Additionally, examination of daughter ions of the ion at m/z 427.5 resulted in disappearance of the ion at m/z 86 seen in Fig. 6, indicating that only background ions unrelated to lidocaine remained in the 427–428 m/z ion peak.

Given that ELISA-based screening is a most commonly used routine method for detection of lidocaine in equine biological samples, we sought to establish a 'detection time' for lidocaine in post-administration urines. In this regard, the local anesthetics are unusual in that they have a very wide clinical dose range, depending on the specific clinical use in question. We, therefore, used a wide range of doses, from a likely sub-therapeutic dose (10 mg) through a low therapeutic dose (40 mg) to relatively high doses such as might be associated with injection of a large joint or a block associated with a field infiltration of this agent.

Note that the ELISA test can react with both lidocaine and its various metabolites; additionally, other unknown compounds in horse urine may interact with the antibody-binding site and alter the optical density of the test wells. ELISA-screening test data, therefore, do not allow one to distinguish between lidocaine or its metabolites or other structurally related compounds in equine biological samples; our ELISA data are therefore reported as apparent lidocaine equivalents.

Analysis of post-lidocaine administration samples by ELISA showed peak urinary concentrations of lidocaine equivalents at 2 h post-administration regardless of dose. The mean peak urinary concentrations of lidocaine were 1.4, 10.2, and 30.4 μg/mL following subcutaneous administration of 10, 40, and 400 mg, respectively (Fig. 8). Apparent lidocaine was still detectable in post-administration urine samples at 96 h following administration (Fig. 9). Apparent mean urinary concentrations of lidocaine were 0.45, 1.73, and 8.6 ng/mL at 96 h following subcutaneous administration of 10, 40, 400 mg of lidocaine, respectively.

In summary, therefore, these results strongly support the hypothesis that the major urinary metabolite of lidocaine in post-administration samples is a hydroxy-lidocaine glucuronide (Harkins *et al.*, 1998). The 427 m/z material found in post-administration urines corresponds with the expected mass of a 3-OH-lidocaine-glucuronide. Daughter ion analysis shows that this material is the parent of 251 m/z and 86 m/z ions, which corresponds with the expected 3-OH-lidocaine and the methylenediethylamino fragmentation products of hydroxy-lidocaine glucuronide. Analysis of the effects of glucuronide hydrolysis on post-administration urines shows that the 427 m/z material disappears following glucuronide hydrolysis, accompanied by the appearance of 251 m/z and 86 m/z fragments. This is consistent with the 427 m/z material being associated with the presence of hydroxylidocaine glucuronide in these post-administration samples. Further work is required to determine whether the principal hydroxylated lidocaine species released by glucuronidase treatment is oxidized in the 3- or 4-position.

Review of the ELISA data suggests that the post-administration ELISA detection time for lidocaine is strongly dependent on the dose administered. In other words, increasing dose results in increased urinary excretion and longer detection time. Additionally, there was a linear correlation between peak mean urinary concentrations and doses of lidocaine as shown in Fig. 8 ($r^2 = 0.947$). Based on the data reported here, when the dose administered is 10 mg/site or less, then the ELISA test does not reveal the presence of lidocaine-related substances by 24 h

post-dosing (Fig. 9). However, if the dose is increased, then there is a significantly increased likelihood of the post-administration sample remaining ELISA positive for 96 h or longer post-dosing. This possibility must be taken into account when estimating 'detection-times' for post-lidocaine administration testing (Fig. 9).

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REFERENCES

Butterworth, J.F.IV & Strichartz, G.R. (1990) Molecular mechanisms of local anesthesia: A review. *Anesthesiology*, **72**, 711–734.

Harkins, J.D., Mundy, G.D., Stanley, S., Woods, W.E., Rees, W.A., Thompson, K.N. & Tobin, T. (1996) Determination of highest no-effect dose (HNED) for local anesthetic responses to procaine, cocaine, bupivacaine, and benzocaine. *Equine Veterinary Journal*, **28**, 30–37.

Harkins, J.D., Mundy, G.D., Woods, W.E. *et al.* (1998) Lidocaine in the horse: its pharmacological effects and their relationship to analytical findings. *Journal of Veterinary Pharmacology and Therapeutics*, **21**, 462–476.

Short, C.R., Flory, W., Hsieh, L.C., Aranas, T. & Barker, S.A. (1988) Metabolism and urinary elimination of lidocaine in the horse. In *Proceedings of the 7th International Conference of Racing Analysts and Veterinarians*. Eds Tobin, T., Blake, J., Potter, M. & Wood, T. International Conference of Racing Analysts and Veterinarians (ICRAV), Lexington, KY.

Soma, L.R. (1971) *Textbook of Veterinary Anesthesia*. Williams and Wilkins, Baltimore.

Tobin, T., Blake, J.W. *et al.* (1977) Pharmacology of procaine in the horse; pharmacokinetics and behavioral effects. *American Journal of Veterinary Research*, **38**, 637–647.

Tobin, T. (1981) *Drugs and the Performance Horse*. Charles C. Thomas, Springfield, IL.

Tobin, T. (1995) Thresholds/limits on testing for therapeutic medications: viewpoints of the regulated. In *Testing for Therapeutic Medications, Enviromental and Dietary Substances in Racing Horses*. Eds Tobin, T., Mundy, G.D., Stanley, S.D., Sams, R.A. & Crone, D.L. The Maxwell H. Gluck Equine Research Center, Lexington, KY.

Voller, A., Bidwell, D.W. & Bartlett, A. (1976) The enzyme linked immunosorbent assay (ELISA). *Bulletin of the World Health Organization*, **53**, 55–56.