Pharmacokinetics of intraperitoneal infusion of lidocaine in horses

by

Joaquin de Estrada, DVM

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Approved by

John Schumacher, Chair, DVM, MS, DACVIM, Department of Clinical Sciences
Jennifer Taintor, Co-chair DVM, MS, DACVIM, Department of Clinical Sciences
Dawn M. Boothe, DVM, MS, PhD, DACVIM, DACVCP, Department of
Anatomy, Physiology, and Pharmacology

Abstract

The objective of this study was to describe the pharmacokinetics of intraperitoneal (IP) lidocaine in horses (30mg/kg). The study was designed as double blinded cross-over, placebo controlled clinical trial, with a 2 weeks washout period. All horses were part of the research herd of the Auburn University Large Animal Teaching Hospital. Four healthy adult, mixed breed horses, 8 to 15 years of age, that weighed between 490 and 570 kg were randomly assigned to receive either placebo or lidocaine first.

A solution of 5 liters of balanced electrolyte solution with or without 2% lidocaine at a dose of 30 mg/kg was injected IP over 20 minutes. Horses were monitored for 24 hours after IP infusion for signs of toxicity. Blood was collected at 0, 5, 10, 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360, 480, and 1440 minutes after infusion. Samples of peritoneal fluid (PF) were obtained at minutes 0, 60, 120, 360 and 1440 minutes. Lidocaine and its active metabolite monoethylglycinexylidide (MEGX) were quantified in plasma and PF using high performance liquid chromatography. Time versus concentration data were subjected to non-compartmental analysis.

Peak plasma (C_{max}) lidocaine and MEGX concentrations were $2.82 \pm 0.84 \mu g/ml$ and $5.58 \pm 3.78 \mu g/ml$, respectively; time to maximum concentration was 75 ± 71 min and 93 ± 98 min respectively. Plasma lidocaine concentration remained above 1 μg/ml for 2 hours and declined to non-quantifiable concentration (< 0.2 μg/ml) by 8 hours after infusion. For IP, C_{max} for lidocaine and MEGX were 2.82 ± 0.84 and 5.58 ± 3.78 μg/ml, respectively. Clinical signs indicative of

lidocaine adversity occurred in one horse after IP administration of lidocaine; this horse recovered completely in 45 minutes without intervention. Further studies are indicated to establish an IP dose of lidocaine necessary to achieve its target effects without causing adverse effects.

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List of Abbreviations

AUC_{∞} Area under the curve extrapolated to infinity

Ca Calcium

Cl Clearance

C_{max} Peak plasma concentration

CN Cranial nerve

CRI Constant rate infusion

GI Gastrointestinal

GX Glycinexylidide

IL-6 Interleukin 6

IP Intraperitoneal

IV Intravenous

LAs Local anesthetics

MEGX Monoethylglycinexylidine

MMC Myoelectric complex

MRT Mean residence time

Na Sodium

PF Peritoneal fluid

PGE2 Prostaglandin E2

POI Postoperatory ileus

T_{max} Time to peak concentration

TNF-α Tumor necrosis factor alpha

$T_{1/2}$Terminal half life

Chapter 1: Literature Review

Brief history of local anesthetics

Lidocaine is an amino-amide compound derived from cocaine. Cocaine, an alkaloid of the shrub *Erythroxylon coca*, is extensively cultivated in the mountain uplands of South America (Peru and Bolivia). Medicinal properties of the coca plant have been known for centuries, and in the Inca Empire the coca plant had a central role in religion. There is archeological proof from the area of the Andes Mountains in South America that coca leaves were harvested and chewed at least 1200 years ago. The stimulating effects of chewing coca leaves are very different to those obtained after consuming cocaine. After the arrival of the Spanish to the Americas, the practice of chewing coca became widespread and was used to increase the productivity of the mine workers and slaves. But it was not until the 19th century that cocaine was refined and introduced to Europe for medical purposes (Gay, et al. 1975). Sigmund Freud and Carl Koller reported the use of cocaine as a local anesthetic for ophthalmologic procedures (Galbis-Reig 2002) and later other physicians reported the use of cocaine for nerve blocks (Gay, et al. 1975). At that time, cocaine had great acceptance in society, as it was introduced in cigarettes, pharmacy formulations, and drinks (e.g. "Vin Mariani", cola sodas) (Gay, et al. 1975). But at the beginning of the 20th century, detrimental cardiovascular effects and dependency-abuse became evident and cocaine was banned by governments around the world. This prompted the development of local anesthetics (LAs) derived from cocaine; the first, procaine, in 1903 (Scholz 2002) and later, lidocaine, during World War II (Ruetsch, Boni and Borgeat 2001). These drugs, in contrast to

cocaine, did not produce detrimental coronary vasoconstriction and myocardial ischemia or infarction associated with administration of cocaine (Weinberg, Current concepts in resuscitation of patients with local anesthetic cardiac toxicity 2002).

Based on their chemical structure, LAs are divided in two classes: amino-esters and amino-amides. Local anesthetics have an intermediate chain that links an amine (hydrophilic) on one end to an aromatic ring (lipophilic) on the other. This chemical structure allows LAs to penetrate a variety of biological membranes. Between the intermediate chain and the aromatic end, amino-amides have an amide link, whereas amino-esters have an ester link. Lidocaine, mepivacaine, bupivacaine, and ropivacaine are classified as amino-amides, while procaine, tetracaine, and benzocaine are amino-esters. Amino-esters are unstable in solution and are metabolized in plasma by pseudocholinesterases. Conversely, amino-amides are stable in solution and are mainly metabolized by hepatic enzymes (McLeod 2011).

Effect of local anesthetics on voltage dependent membrane ion channels

Lidocaine, an amino-amide local anesthetic, is widely used in veterinary medicine for perineural and intrarticular anesthesia (Dickey, et al. 2008). Local anesthetics prevent and relieve pain by interrupting nerve excitation and conduction in a reversible, direct interaction with voltage-gated sodium (Na) channels that block membrane electrical excitability. Most amino-amide LAs are ionized at a pH of 6, which facilitates their diffusion through the cell membrane. Once inside the cell, LAs bind selectively to the α-subunit of open voltage-gated, transmembrane

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Na channels with positively charged terminal amino acids. Binding of LAs to the Na channel maintains the channels open, which allows Na molecules to diffuse freely to into the cell and prevents the cell membrane from depolarizing. In consequence, transmission of the excitatory axonal ion conductance is blocked. Voltage-gated Na channels are found primarily in the nodes of Ranvier in myelinated axons, where they provide saltatory nerve conduction (England, et al. 1990).

Lidocaine is also used to treat cardiac arrhythmias and it was first reported to be used as a cardiac antiarrhythmic in 1950. Because of its action on Na channels, lidocaine causes a slight decrease in cardiac automaticity (spontaneous phase 4 depolarization) of pacemaker cardiac cells. In humans, lidocaine administered intravenously (IV) is highly effective in terminating ventricular premature beats and ventricular tachycardia occurring during general anesthesia, during and after cardiac surgery, following acute myocardial infarction, and in the treatment of digitalis intoxication (Collinsworth, Kalman and Harrison 1974).

Different isoforms of Na-channels have been described, two are peripheral nerve isoforms: a skeletal muscle isoform, and a cardiac isoform (Lipkind and Fozzard 2005); however, not all the properties of LAs can be attributed to the blockage of Na channels. It was proposed that undesirable side-effects on the transmission of impulses in nerve synapses of the brain and cardiac muscle cells could be related to the action of LAs on K voltage-independent and calcium (Ca) voltage-dependent channels present in C-nerve fibers of the horn of the spinal cord. In addition to the effect on ion channels, LAs might have an intracellular effect on G-

proteins and G-protein regulated channels, which could explain unexpected effects on other organs (Scholz 2002).

Analgesic effects of lidocaine administered systemically

Intravenous administration of lidocaine has been successfully used to treat central pain in humans (Carroll 2007). In the amplification of a painful stimulus, the expression or activity of voltage-gated Na channels is increased by interaction of inflammatory mediators, such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and prostaglandin E2 (PGE2) with nerve growth factor that results in magnification of neuronal excitability (Wood, et al. 2004). In a double-blind, placebo-controlled, and cross-over study that involved human patients suffering from central neuropathic pain, lidocaine was superior to saline in reducing the intensity of pain attributable to stroke or spinal cord injury. It was proposed that these effects were mediated by selective suppression of C-fiber nerves of the spinal cord and hyperexcitable dorsal horn neurons (Attal, et al. 2000). Perioperative, systemically administered lidocaine significantly reduced opioid requirements after ambulatory surgery, in people. These results were attributed to inflammatory modulatory properties of lidocaine (McKay, et al. 2009).

In an experiment with horses, systemically administered lidocaine produced thermal antinociception, but minimal changes in visceral nociception (Robertson, et al. 2005). However, a study that evaluated the electroencephalogram (EEG) of ponies anesthetized with halothane for castration concluded that IV administration of lidocaine perioperatively was antinociceptive and

contributed to anesthesia during castration. These researchers compared the EEG reported for ponies during castration with those that also received IV lidocaine. Administration of lidocaine decreased the intensity of the EEG changes compared to ponies that did not receive lidocaine, suggesting that lidocaine prevented a cortical response to castration (Murrell, et al. 2005).

Intravenous lidocaine for treatment of postoperative ileus

Systemic administration of lidocaine postoperatively was shown to shorten the duration of ileus and postoperative recovery after abdominal surgery in people (Marret, et al. 2008) and horses (Cook and Blikslager 2008, Malone, et al. 2006, Brianceau, et al. 2002, Torfs, et al. 2009). In an ultrasonographic study that evaluated the effects of IV lidocaine on small intestinal size, quantity of peritoneal fluid, and outcome after colic surgery, horses that received lidocaine had significantly smaller minimum jejunal cross-sectional area scores, minimum jejunal diameter scores, and less intestinal abnormalities (Brianceau, et al. 2002). However, the authors reported no significant differences between the groups for the presence of gastrointestinal (GI) sounds, time to passage of first feces, number of defecations in the first 24 hours, presence of gastric reflux, duodenal or jejunal wall thickness, maximum duodenal or jejunal diameter or cross-sectional area, minimum duodenal diameter or cross-sectional area, duodenal and jejunal intraluminal echogenicity, small-intestinal contractions per minute, rate of complications, or survival. Remarkably, the entire population of this study had a low incidence of gastric reflux after surgery and there were few horses with small intestinal lesions in the study, which could have skewed the results.

Another study observed a population of 32 horses that were diagnosed with postoperative ileus (POI) or enteritis and produced large volumes of reflux (Malone, et al. 2006). In this study 65% of the horses treated with lidocaine stopped refluxing within 30 hours, whereas 27% of the control group stopped in the same amount of time and horses treated with lidocaine passed feces sooner than the controls. The lidocaine treatment resulted in shorter hospitalization time for survivors, when compared with placebo

In a retrospective, cross-sectional study of 126 coliky horses that underwent surgery for small intestinal disease, a group of researchers assessed the association of lidocaine treatment with short-term survival (Torfs, et al. 2009). In that study prophylactic lidocaine treatment was significantly associated with a reduced incidence of POI and increased likelihood of short-term survival. These researchers concluded that the evidence in their study supported the use of lidocaine for prokinesis after small intestinal colic surgery; however, they speculated that anti-inflammatory properties of lidocaine, rather than prokinetic effects could be responsible for the positive outcome of horses with POI treated with lidocaine.

Lidocaine was the most common drug used to treat POI in horses in a survey of Diplomates of the American College of Veterinary Surgeons (Hoogmoed, et al. 2004), but there is still significant uncertainty about its specific mechanism of action (Cook and Blikslager 2008). Researchers showed that lidocaine increased the contractile activity of smooth muscle collected from the proximal portion of the duodenum of horses. The effect of lidocaine on smooth muscle contractility was attributed to a suppression of inhibitory reflexes, active during bowel and peritoneal inflammation; however, because GI motility is a complex process that involves

muscular, neural, and humoral factors, as noted by the study's authors, the effects of lidocaine *in vivo* could differ from the results in this *in vitro* study (Nieto, Rakestraw and Snyder 2000). Other researchers also proposed that lidocaine had a direct positive effect on GI peristalsis (de Solis and McKenzie 2007, Torfs, et al. 2009, Koenig and Cote 2006, Cohen, et al. 2004), but this information was based on the clinical and experimental observation that lidocaine decreased the severity and duration of POI in horses, rather than in results of controlled studies on GI peristalsis (Dickey, et al. 2008). Furthermore, lidocaine was still considered a prokinetic agent in review articles (Koenig and Cote 2006, Hudson and Merrit 2008) and textbook chapters (Lester 2009, Sanchez 2010), but the experimental evidence to support the peristaltic effect of LAs is scarce (Cook and Blikslager 2008).

A study that researched the effectiveness of prophylactic administration of lidocaine for equine POI confirmed previous findings (Malone, et al. 2006) that IV administration of lidocaine has a positive effect on short-term survival. Furthermore, the positive effect of lidocaine administration on survival was significant in a multivariable model, independent of POI. These researchers questioned the prokinetic mechanism of action of lidocaine in horses with POI and proposed that various other properties of lidocaine, such as anti-inflammatory, analgesic, and anti-endotoxin effects, likely mediated the positive effect of lidocaine on short-term survival (Dickey, et al. 2008).

Recent *in vivo* research also challenged the concept of prokinetic properties of lidocaine. A study evaluated the effect of lidocaine on the duration of the migrating myoelectric complex (MMC) and Phases I, II, and III of the MMC, spiking activity of the jejunum, and number of

Phase III events when administered to horses subjected to celiotomy (Milligan, et al. 2007).

These researchers reported no differences between horses treated with lidocaine and controls. In

another study that evaluated intestinal motility of horses after administration of different drugs,

researchers concluded that IV lidocaine did not affect jejunal or cecal motility (Okamuraa, et al.

2009). In conclusion, the evidence presented by these studies failed to support the proposed

prokinetic mechanism of lidocaine on horse with POI. Furthermore, a study that evaluated the

fecal transit time of healthy horses that received barium filled microspheres orally showed that

horses that received lidocaine IV had a slower GI transit time than the controls (Rusiecki, et al.

2008). The evidence of the afore mentioned studies indicates that IV lidocaine may have a

positive effect on horses after abdominal surgery, but it is likely that the effectiveness of

lidocaine is due to mechanisms of action other than a direct effect on the contractibility of

intestine (Cook and Blikslager 2008).

Anti-inflammatory properties of local anesthetics

There is broad evidence indicating anti-inflammatory effects of LAs in people and

domestic animals, including horses (Caracas, et al. 2009, Cook and Blikslager 2008, Hollmann

and Durieux 2000, MacGregor, Thorner and Wright 1980, McCafferty, Sharkey and Wallace

1994). Several authors characterized anti-inflammatory effects of lidocaine on neutrophils,

independently of the effect on Na channels (Ando, et al. 2009, Azuma, et al. 2000, Cook, et al.

2009, Hyvonen and Kowolik 1998, Jinnouchi, et al. 2005, Kidd and Urban 2001, Lan, et al.

2004, MacGregor, Thorner and Wright 1980, Mikawa, et al. 1997).

In a review article, the anti-inflammatory properties of LAs were summarized in 9 categories (Hollmann and Durieux 2000). The authors of the review cited evidence from *in vitro* and *in vivo* studies that documented the effects of LAs on the release of inflammatory mediators, adhesion, migration, accumulation, and priming of neutrophils, free radical and granule enzyme release, lysosomal enzyme release, and nitric oxide generation, as well as inhibition of the respiratory burst in macrophages. Interestingly, the authors of the review concluded that in most *in vitro* studies, concentrations of LAs above the clinically relevant range were required for these effects. In addition, results of *in vitro* studies using post-ischemic reperfusion models indicated that anti-inflammatory effects of lidocaine on human neutrophils appear to be dose dependent and more effective at high concentrations (2-8 mg/ml) (Hyvonen and Kowolik 1998). In another study, reactive oxygen species were inhibited in human neutrophils with lidocaine concentrations over 20 μg/ml (Mikawa, et al. 1997). Furthermore, lidocaine was found to be more effective as an anti-inflammatory drug when administered topically (up to 100 mg/kg) rather than systemically, in models of experimental colitis (McCafferty, Sharkey and Wallace 1994) and also in clinical cases of colitis (Yokohama and Onishi 2005). These observations were crucial for design of our project, because the IP route of administration of lidocaine allowed administration of lidocaine at a dose 23 times higher than the dose commonly recommended (Malone, Turner and Wilson 1999) for IV administration in horses. We postulated that a high dose of lidocaine administered IP could provide horses suffering from intestinal disease with anti-inflammatory effects not previously observed. However, an *in vitro* model evaluated the migration and adhesion of equine neutrophils

incubated in increasing concentrations of lidocaine (Cook, et al. 2009). In contrast to experiments that used human neutrophils, in which lidocaine inhibited neutrophil migration or adhesion *in vitro*, lidocaine had the opposite effect on equine neutrophils, increasing migration and adhesion at the highest concentration (1 mg/ml). Nonetheless, *in vitro* models ignore many variables that affect lidocaine, such as blood pH, ionization of the drug, binding to serum proteins, and possibly other factors (McLeod 2011).

Because lidocaine has inhibitory effects on neutrophil adhesion, phagocytosis, and the production of free radicals, it was proposed that the beneficial effect of lidocaine in the treatment of horses with ileus could be due to reduction of the deleterious effect of neutrophils on intestinal contractibility (Cook and Blikslager 2008). The inhibitory effect of lidocaine on neutrophil migration to the intestinal wall was also proposed for its beneficial effect for treatment of intestinal ischemic-reperfusion injuries in horses (Cook et al. 2009). An *in vivo model* that used horses with ischemia-reperfusion injury of the jejunum showed that horses that received IV lidocaine had reduced plasma PGE2 metabolite concentration and mucosal cyclooxygenase 2 (COX-2) expression when compared to controls. Administration of lidocaine with flunixin meglumine ameliorated an increase in mucosal neutrophil counts induced by flunixin meglumine alone (Cook, et al. 2009).

Pharmacokinetics of lidocaine administered intravenously to horses

A plasma lidocaine concentration between 1 and 2 μg/ml was proposed to be effective for the treatment of horses with POI and anterior enteritis (Malone, Turner and Wilson 1999). This plasma concentration was referred as the "target concentration" in subsequent reports (de Solis and McKenzie 2007, Brianceau, et al. 2002, Milligan, et al. 2006). Other studies confirmed a similar lidocaine plasma concentration in horses with POI that were successfully treated (Malone, et al. 2006, Brianceau, et al. 2002); however, a significant variability in plasma lidocaine concentrations was also reported when similar doses of lidocaine were administered to horses (de Solis and McKenzie 2007, Milligan, et al. 2006, Brianceau, et al. 2002). To reach the target concentration, it was recommended that lidocaine be administered at a loading dose of 1.3 mg/kg followed by a continuous rate infusion (CRI) of 0.05 mg/kg/min (Milligan, et al. 2006, Cook and Blikslager 2008). In postoperative horses that had undergone celiotomy, this dose produced lidocaine plasma concentrations of 1.21– 3.13 μg/ml (n=6) (Milligan, et al. 2006).

In a study that used healthy horses (n=8), serum concentrations of lidocaine and its metabolites were evaluated after four days of infusion (Dickey, et al. 2008). These researchers used a CRI of lidocaine at 0.05 mg/kg/min that produced a mean concentration of 0.938 ± 25.2 μg/ml. Lidocaine reached steady state concentration by 3 hours after infusion, decreased quickly after discontinuation of the infusion and was below detectable limits 24 hours later. In contrast to design of other studies, these researchers elected not to use a loading dose in all horses. When horses received the bolus dose, lidocaine plasma concentrations remained below the limit of

detection, thus the authors concluded that any influence of the bolus on long term infusions would have been very transient.

The pharmacokinetics of lidocaine in horses appears to be affected by many factors associated with the postoperative period. One study evaluated horses that had undergone abdominal surgery 72 hours prior to infusion of lidocaine. Horses were pre-medicated with flunixin meglumine (1.1 mg/kg IV), they were induced to anesthesia with xylazine (1.1 mg/kg IV) and ketamine (2.2 mg/kg IV) and maintained with guaifenesin (100 mg/kg/h), ketamine (2 mg/kg/h), and xylazine (1 mg/kg/h), administered for 40–60 min. Lidocaine was administered as a bolus dose of 1.3 mg/kg followed by a CRI of 0.05 mg/kg/min over 12 hours. Plasma lidocaine concentrations (1.21–3.13 µg/ml) were higher in this study than in other studies that used the same dosing protocol. However, it may be significant that these researchers used a study protocol similar to a clinical situation where the effect of general anesthesia, or administration of other drugs (flunixin meglumine in particular) could have affected their results. These authors concluded that lidocaine may need to be administered at a lower rate to horses when long-term treatment with lidocaine is anticipated.

Other authors obtained similar results using slightly different regimens of administration. Robertson et al. administered lidocaine as a bolus of 2 mg/kg over 20 minutes followed by a CRI of 0.05 mg/kg/min that produced a maximum concentration (C_{max}) of 0.92 ± 0.06 µg/ml at the end of the bolus injection and remained constant during the infusion (n=6) (Robertson, et al. 2005). Brianceau *et al.* (2002) administered lidocaine IV after induction of anesthesia (n=14), with a loading dose of 0.65 mg/kg, followed by a CRI of (0.025 mg/kg/min). Upon recovery

from anesthesia, these researchers administered a second loading dose of 1.3 mg/kg, followed by an infusion of 0.05 mg/kg/min for 24 hours. The results of this study showed a broad variation of lidocaine plasma concentrations, ranging from 0.15 to 2.72 μg/ ml, with a mean concentration of 1.02 ± 0.32 μg/ml. The intraoperative lidocaine concentrations ranged from a minimum of 0.39 to 2.72 μg/ml, with a mean of 1.06 ± 0.6 μg/ml. Postoperative concentrations ranged from 0.15 to 2.16 μg/ml, with a mean of 1.0 ± 0.52 μg/ml.

de Solis and McKenzie (2007) administered a bolus of lidocaine (0.8-1.3 mg/kg) followed by a CRI of 0.05 mg/kg/min to a group of horses after colic surgery (n=8) while the horses were anesthetized. After returning to the stall, the horses received a second loading dose (1.3 mg/kg IV over 15 minutes) followed by 0.05 mg/kg/minute intravenous CRI. Plasma concentrations of lidocaine in these horses were below the target concentrations (1-2 μg/ml) after the immediately, postoperatively administered loading dose, but reached therapeutic concentrations after 6 hours of CRI which remained constant for approximately 12 hours. These researchers also reported significant variability of lidocaine plasma concentrations between individuals and sampling time.

There is a high variability in plasma lidocaine concentrations within and between studies (de Solis and McKenzie 2007, Milligan, et al. 2006, Brianceau, et al. 2002, Valdeverde, et al. 2005). This variability is not surprising, considering all the variables that have been reported to affect the pharmacokinetics of lidocaine, such as renal function, fasting (Engelking, et al. 1987), acid base status (Yakatis, Thomas and Mahaffey 1976), albumin binding capacity (Milligan, et al. 2006), liver function (Orlando, et al. 2004), cardiac output (Collinsworth, Kalman and

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Harrison 1974), general inhalation anesthesia (D. J. Feary, et al. 2005), GI disease (D. Feary, et al. 2006) and endotoxic shock (McKindley, et al. 2002).

General anesthesia has a profound influence in the disposition of lidocaine in horses. A study compared the pharmacokinetics of lidocaine in conscious and anesthetized horses that received a lidocaine bolus of 1.3 mg/kg followed by a CRI of 0.05 mg/kg. The mean serum lidocaine concentrations of conscious horses (1.84 ± 0.38 μg/ml) were significantly less than those in anesthetized horses (3.34 ± 0.6 μg/ml) during loading and maintenance infusion periods in this study. When the disposition of lidocaine was compared between conscious and anesthetized horses, researchers reported that anesthetized horses had higher Cmax and area under the curve (AUC), likely due to changes in the volume of distribution (VDss) and clearance (Cl) of the drug (Rezende, et al. 2011). They proposed that these differences were caused by a decrease in cardiac output and hepatic blood flow in anesthetized horses, probably caused by administration of xylazine and sevoflurane. (D. J. Feary, et al. 2005). After administering the same dose of lidocaine, other researchers obtained similar plasma concentrations (2.58 ± 0.81 μg/ml) in horses anesthetized with sevoflurane. In an effort to compensate for the effect of anesthesia on the pharmacokinetics of lidocaine, other researchers decreased the dose of lidocaine administered to anesthetized horses by 50 % (0.65 mg/kg bolus, 0.025 mg/kg/min CRI) (Brianceau, et al. 2002).

The influence of GI tract disease on pharmacokinetics of IV lidocaine in anesthetized horses was described in a study that evaluated a group of horses that underwent celiotomy for GI disease (D. Feary, et al. 2006). Data regarding the disposition of lidocaine obtained in that study

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was similar to data obtained by the same researchers from horses anesthetized for treatment of orthopedic disease that received lidocaine with a similar dosing regimen. Horses with GI disease had lower plasma lidocaine concentrations, and higher distribution and total body clearance (Vdss 0.7 ± 0.31 L/kg; CL 25 ± 3 ml/min/kg), compared with concentrations found in healthy anesthetized horses (Vdss 0.40 ± 0.09 L/kg; CL 15 ± 3.3 ml/min/kg) determined in a previous study (D. J. Feary, et al. 2005) by the same research group. These differences were attributed to tachycardia and higher cardiac output in horses with GI disease, when compared to healthy horses. It is interesting that in this study horses were exposed to variables that are common in the clinical setting (fasting, administration of antibiotic and anti-inflammatory drugs) when a horse undergoes abdominal surgery for treatment of GI disease. However, it is also possible that many of these variables could have affected the disposition of lidocaine and the association of GI disease could be confounded by management or drugs used to treat a horse with colic. For example, all the horses in this study were fasted before surgery, which has been reported to alter the distribution of lidocaine (Engelking, et al. 1987). They also received flunixin meglumine, which has been reported to displace lidocaine from albumin binding sites and therefore, increase its availability in plasma (Milligan, et al. 2006).

The effect of endotoxic shock on the distribution of lidocaine was determined in a murine model (McKindley, et al. 2002). Serum concentrations of lidocaine were higher in rats with endotoxic shock versus control rats and MEGX (lidocaine's main metabolite) concentrations were significantly higher in rats with endotoxic shock versus control rats. These findings were primarily attributed to reduced hepatic blood flow and secondarily to impaired activity of

CYP450, one of which was CYP3A4, a key hepatic enzyme in the metabolism of lidocaine. These authors also concluded that the ratio of MEGX to lidocaine concentrations decreased significantly during endotoxic shock and could be useful as a measure of hepatic function during endotoxic shock.

Because of its profound effect on inhalation anesthetic drugs, lidocaine has been administered during anesthesia to decrease the volume of inhalation anesthetics administered to minimize their cardiovascular and respiratory depressant effects. Intravenous lidocaine reduced the halothane minimum alveolar concentration (MAC) in ponies in a dose-related manner (Rezende, et al. 2011). Ponies were given a bolus injection of 2.5 or 5 mg/kg of lidocaine followed by a CRI of 0.05 (low dose) to 0.1 mg/kg/min (high dose) during anesthesia with halothane. Serum lidocaine concentrations varied between 1-4 μg/ml and 3-7 μg/ml, for the low and high dose, respectively, which correlated with an inversely proportional reduction of halothane MAC (up to 50-70%) (Doherty and Frazier, Effect of intravenous lidocaine on halothane minimum alveolar concentration in ponies 1998). In a study of horses that underwent anesthesia for elective procedures, IV administration of lidocaine at 2.5 mg/kg bolus and 0.05 mg/kg/min CRI, resulted in a 25% reduction in isoflurane requirement (Dzikiti, Hellebrekers and Dijk 2003).

In a group of healthy horses (n=8) anesthetized with sevoflurane, IV administration of lidocaine at a bolus dose of 1.3 mg/kg followed by a CRI of 0.05 mg/kg/min produced a mean MAC reduction of $26.7 \pm 12\%$ (Rezende, et al. 2011). Plasma lidocaine concentrations were

similar to those reported in anesthetized horses, but still higher than those concentrations usually obtained in conscious horses. At the end of the bolus, plasma lidocaine concentrations were 2.58 ± 0.81 μg/ml and remained between 2.06 ± 0.44 μg/m and 2.25 ± 0.21 μg/ml. In this study, lidocaine infusion was discontinued approximately 15 minutes before the end of anesthesia and anesthetic recoveries were considered satisfactory. In contrast to these results, a study on horses anesthetized with sevoflurane or isoflurane, reported that the horses administered lidocaine intravenously (bolus 2 mg/kg, CRI 0.05 mg/kg) until the end of surgery had a significantly higher degree of ataxia and a tendency towards significance for a lower quality of recovery when compared to horses that received lidocaine CRI until 30 minutes before the end of surgery (Valdeverde, et al. 2005). These researchers found no correlation between lidocaine plasma concentrations at recovery and the quality of recovery. Other studies reported uneventful recovery from inhalation anesthesia after administration of similar doses of lidocaine during anesthesia (Dzikiti, Hellebrekers and Dijk 2003, Doherty and Frazier, Effect of intravenous lidocaine on halothane minimum alveolar concentration in ponies 1998), but in contrast to the study of Valverde et al, the dose of inhalation anesthetic in these studies was decreased by 25%. Furthermore, in another study horses that received a combination of lidocaine (1.3 mg/kg bolus, 0.04 mg/kg/min CRI) and ketamine (0.06 mg/kg/min CRI) had a reduction of 65% of the initial isoflurane end-tidal concentration (Enderle, Levionnois and Kuhn 2008). In this study, no difference was noted in the quality of the recovery of lidocaine-treated horses from anesthesia, when compared to the control group.

An *in vitro* study evaluated the plasma protein binding of lidocaine in horses. Because only unbound lidocaine is pharmacologically active, highly protein bound drugs could displace lidocaine from plasma protein and lead to higher plasma concentrations and therefore, signs of toxicity. It is also possible that drugs with high extraction, as is lidocaine, can be eliminated faster from plasma when displaced from protein binding sites. Results of this study revealed that plasma protein binding in horses was 53%, which indicates that 47% of lidocaine injected IV would be available to elicit a pharmacological effect. Intravenous administration of flunixin meglumine increased the unbound lidocaine concentration by 17%, while ceftiofur produced an increase of unbound lidocaine concentration of 26%. The combined administration of these two commonly used drugs could increase the amount of lidocaine available by 50%, despite causing no change in total plasma concentrations (Milligan, et al. 2006). This observation is of remarkable importance to interpret the pharmacokinetics of lidocaine in horses treated with other drugs and could explain, to some extent, the variability observed between studies. However, it is challenging to extrapolate or compare the results of *in vitro* studies to *in vivo* studies. Concern was expressed by other authors about the potential effect of concomitant administration of drugs that affect hepatic metabolism, such as erythromycin and fluoroquinolones, on the pharmacokinetics of lidocaine (Dickey, et al. 2008). But this concern is not supported by experimental evidence in horses.

Distribution of lidocaine administered intraperitoneally

Although there are no reports of IP administration of lidocaine in horses, several studies that used human and animal models determined the distribution of lidocaine after IP administration. Amino-amide LAs have been safely administered IP to rats (Brocco, et al. 2008, Yuzbasioglu, et al. 2008), rabbits (Rimback, et al. 1988), dogs (Wilson, Barnes and Hauptman 2004), and people (Ali, et al. 1998, Klein 1990, Narchi, et al. 1992, Ng and Smith 2002, Williamson, Cotton and Smith 1997, Chundrigar, et al. 1993, Carroll 2007, Elhakim, et al. 2000).

Safety and pharmacokinetics of lidocaine were determined in dogs that received a combination of lidocaine IP (8 mg/kg with epinephrine 1:400 000) and in the abdominal incision (2 mg/kg with epinephrine 1: 200 000) following ovariohysterectomy (Wilson, Barnes and Hauptman 2004). Lidocaine was rapidly absorbed (0.37 ± 0.26 h) and produced a peak plasma concentration of 1.45 ± 0.36 μg/ml, with an AUC of 2.96 ± 0.45 μg/ml. The absorption half-life was 0.13 ± 0.1 h and the elimination was 1.17 ± 0.11 h, but it is possible that the distribution of lidocaine in this study was affected by the use of lidocaine combined with epinephrine. This study reported that no signs of toxicity were observed in these dogs after drug administration.

In a study in women that underwent elective diagnostic laparoscopy, researchers administered a total of 400 mg of lidocaine IP per patient, using lidocaine, or two formulations of lidocaine with adrenaline (1/320.000 and at 1/800.000) (Narchi, et al. 1992). Women administered lidocaine without adrenaline had higher plasma lidocaine concentrations and absorbed lidocaine faster than those treated with lidocaine and adrenaline in combination. This

study also reported no signs of toxicity. In another study, women that underwent postpartum tubal ligation, received 550 mg of lidocaine IP, which produced a maximum plasma lidocaine concentration of 2.7 µg/ml similar to previous similar studies (Visalyaputra, et al. 1999). Again, no signs of toxicity were reported. In a report that described the use of lidocaine administered IP for liposuction, doses as high as 35 mg/kg were administered (Klein 1990). This research examined the absorption of dilute solutions of lidocaine (0.1% or 0.05%) and epinephrine (1:1,000,000) in physiologic saline following infiltration into subcutaneous fat of liposuction surgery patients. This author concluded that by diluting lidocaine the peak plasma lidocaine concentrations were diminished and delayed, thereby reducing potential toxicity. Peak plasma lidocaine concentrations ranged between 0.8 and 2.7 µg/ml over a dosage range of 11.9-34.1 mg/kg

Metabolism of lidocaine

Following systemic administration, lidocaine has a high hepatic extraction rate, as it is metabolized mainly in the liver via oxidative N-dealkylation by the cytochrome P450 system (CYP) to monoethylglycinexylidine (MEGX) (Alexson, et al. 2002). In people, CYP-1A2 is a major microsomal enzyme system determinant of lidocaine metabolism *in vivo* (Orlando, et al. 2004), whereas CYP-2B1 and 3A2 are the major P450 isoforms in rats (Nakamoto, et al. 1996). MEGX is then further metabolized to glycinexylidide (GX) and other minor metabolites that are later excreted with the urine (Collinsworth, Kalman and Harrison 1974).

Total body clearance of lidocaine (25-29 ml/min/kg) approximates the hepatic blood flow (D. J. Feary, et al. 2005). Therefore, it has been proposed that changes in the portal circulation could directly affect the hepatic metabolism of lidocaine (Engelking, et al. 1987). Furthermore, fluctuations of the cardiac output (e.g. anesthesia, tachycardia), could cause variations in hepatic circulation and, in consequence, in the metabolism of lidocaine (Milligan, et al. 2006, Dickey, et al. 2008).

Lidocaine is formulated as a weak base (pKa' =7.85) and 3-10% of lidocaine in unchanged form may be excreted in the urine, depending on the urinary pH (Dickey, et al. 2008), along with its metabolites (Collinsworth, Kalman and Harrison 1974). In horses, a linear correlation between peak mean urinary concentrations and doses of lidocaine was described in a study designed for drug testing in competitions, but the test used in this study (ELISA) did not differentiate between lidocaine and its metabolites (Dirikolu, et al. 2000). It was reported that renal disease has no influence on the elimination of lidocaine and MEGX; in contrast GX may accumulate in the presence of renal dysfunction (Dickey, et al. 2008).

Because metabolism of lidocaine depends primarily on the liver, serum concentrations of lidocaine and MEGX have been used in human medicine to assess hepatic function before organ transplant and in patients with chronic hepatitis and cirrhosis (Forman and Lucey 2003, Ercolani, et al. 2000). The procedure consists of determination of MEGX 15 minutes after injection of lidocaine IV (1 mg/kg). MEGX plasma concentrations above 0.09 μg/ml are expected in liver donors (Ercolani, et al. 2000). Conversely, other research evaluated the extrahepatic conversion of lidocaine to MEGX using a model of liver bypass in rats (Ping, et al. 2001). Results of this

study revealed that serum total MEGX in the model group was $32.0 \pm 7.14\%$ of that in the control group. Previous administration of phenobarbital decreased conjugated MEGX significantly and increased free MEGX increased by 167.9%. The importance of extrahepatic metabolism of lidocaine in people was considered clinically insignificant (Ercolani, et al. 2000). The importance of extrahepatic metabolism of lidocaine could be different for other animal species. Extrahepatic metabolism of lidocaine occurs in respiratory and upper GI tract of rats, but enzymes of the CYP-450 family were also detected in other organs of different animal species (Tjalve and Larsson 2006).

Lidocaine's major metabolites: GX and MEGX

Metabolites of lidocaine in the horse include 3-hydroxylidocaine, dimethylaniline, 4-hydroxydimethylaniline, MEGX, 3-hydroxymonoethylglycinexylidine and GX (Harkins, et al. 1998) . Although lidocaine disappears quickly from plasma, MEGX and GX accumulate in the plasma of horses during prolonged infusions. In healthy horses (n=8), serum concentrations of lidocaine and its metabolites were evaluated in a study after prolonged infusion (4 days). In this study, serum MEGX concentrations reached an apparent steady state concentration of ~0.45 µg/ml by 6 hours after infusion and did not increase significantly after this time. Another main lidocaine metabolite, GX, appeared to accumulate over time. Mean concentration of GX increased from 0.368 µg/ml at 3 hours to 1.226 µg/ml at 48 hours after infusion (Dickey, et al. 2008).

Both main metabolites of lidocaine, MEGX and GX have pharmacological activity, but their effects have not been characterized in horses. At high concentrations, MEGX and GX produced convulsions and death in rats (Blumer, Strong and Atkinson 1973) and it was postulated that MEGX could contribute to the total anti-arrhythmic effect of lidocaine infusion (Burney, et al. 1974, Kates 1984).

Therapeutic properties of lidocaine for peritonitis

<u>Anti-inflammatory</u>

Amino-amide LAs are well tolerated in the abdomen of rats (Brocco, et al. 2008, Yuzbasioglu, et al. 2008), rabbits (Rimback, et al. 1988), dogs (Wilson, Barnes and Hauptman 2004), and people (Ali, et al. 1998, Klein 1990, Narchi, et al. 1992, Ng and Smith 2002, Williamson, Cotton and Smith 1997, Chundrigar, et al. 1993, Carroll 2007, Elhakim, et al. 2000).

Recent research indicates that anti-inflammatory properties of lidocaine may be of value in treatment of animals with peritonitis. Peritonitis was induced in rats by IP infusion of their own feces and 6 hours later the rats were subjected to laparotomy (Brocco, et al. 2008). The abdomen was lavaged and the fluid was aspirated in one group; in another group the abdomen was lavaged with saline and then dried; and in a different group, the abdomen was lavaged with a solution that contained lidocaine (30 mg/kg) and dried. Bacteria isolated from the peritoneal fluid (PF) collected during laparotomy included *Proteus mirabilis, Klebsiela pneumoniae, Enterococus faecalis, Escherichia coli, Micrococus, Proteus penneri, Enterococus gallinarum, Staphylococcus sciuri, Bacillus species, Staphylococcus epidermidis, Aerococcus viridians.* Rats

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in the control group and those that had the fluid in the abdomen aspirated during laparatomy showed signs of sepsis and died in less than 52 hours. Fifty percent of the rats that received an abdominal lavage with saline survived, which was interpreted as a beneficial effect of abdominal lavage in this model. Interestingly, all rats whose abdomen was lavaged with lidocaine survived and had no signs of sepsis after11 days. Whether the results of this study were due to local or systemic effects of lidocaine was unclear to the authors. However, in this model IP infusion allowed administration of a high dose of lidocaine (30 mg/kg) that if administered systemically would likely have caused signs of toxicity.

In another study lidocaine was administered subcutaneously for 7 days to rats in a model of peritonitis caused by cecal ligation and puncture (Gallos, et al. 2004). Compared to rats in the control group all of which died, administration of lidocaine reduced mortality to 0%. Furthermore, proinflammatory mediators (TNF-α, keratinocyte- derived chemokine, ICAM-1 protein expression), renal and hepatic biochemistry parameters and histology findings, and renal cells apoptosis were significantly lower in the rats that received lidocaine. Administration of lidocaine in this study attenuated the hyperacute inflammatory response, which resulted in improved mortality and attenuated signs of sepsis, as reflected in the attenuated progression to acute kidney and liver injury and dysfunction.

The effect of topical lidocaine and bupivacaine was evaluated in rats exposed to a solution of hydrochloride acid (HCl) administered intraperitoneally (Rimback, et al. 1988). Peritonitis was quantified by extraction of Evans blue-bound albumin by measuring albumin extravasation in the peritoneum. In rats that received lidocaine before or after HCL, Evans blue

albumin extravasation did not differ significantly from the rats not receiving HCl (controls). Results were interpreted as a significant inhibition of peritonitis in the groups treated with lidocaine. In these experiments, lidocaine was reported to have a stronger anti-inflammatory effect than bupivacaine. In addition, the penetration of LAs into the tissue was visualized by autoradiography and both the inflammatory reaction and lidocaine were detected only on the serosa layer of the intestines. Anti-inflammatory effects of lidocaine were considered stronger than methylprednisolone in a study that evaluated adherence and delivery of inflammatory cells after induction of peritonitis in rabbits (MacGregor, Thorner and Wright 1980). In this model, lidocaine administered IV caused a more than10-fold greater inhibition of inflammation in the peritoneum than did methylprednisolone administered intravenously.

Local anesthetics could prevent formation of peritoneal adhesions caused by bacterial peritonitis. Rats that had peritonitis induced by ligation and puncture of the cecum were treated intraperitonally with a combination of lidocaine and prilocaine (EMLA) (Yuzbasioglu, et al. 2008). Tissue anti-oxidant levels in abdominal tissue (superoxide dismutase, catalase, lipid peroxidation) were measured. Bacteria isolated from the abdominal cavity of these rats included *Escherichia coli, Enterobacter aerogenes, Proteus mirabilis, Proteus vulgaris,* group D *Streptococcus, Enterococcus, Staphylococcus aureus, Clostridium difficile,* and *Bacteroides fragilis.* A significantly lower score of adhesions and anti-oxidant enzymes were detected in the abdomen of the group that received EMLA, compared with a group that received only lidocaine and a control group.

The effects of lidocaine infusion during experimental endotoxemia were evaluated in horses using a model of peritonitis (Peiro´, et al. 2010). Twelve healthy horses received a dose of lipopolysaccharide in the abdomen and were treated with IV lidocaine or saline. Compared with the control group, horses treated with lidocaine had significantly milder clinical signs of endotoxemia, as well as a decrease in serum and PF TNF-α activity by 3-fold. Contrary to findings in other species (MacGregor, Thorner and Wright 1980), lidocaine did not attenuate leukocyte migration into the abdominal cavity of horses in this study.

Analgesia of local anesthetics administered intraperitoneally

The analgesic effect of lidocaine administered IP is a topic of controversy in human medicine; however, most studies agree that IP administration of lidocaine is apparently safe (Williamson, Cotton and Smith 1997, Narchi, et al. 1992, Ali, et al. 1998, Elhakim, et al. 2000, Visalyaputra, et al. 1999, Boddy, Mehta and Rhodes 2006). In a pilot study that evaluated pain in women after hysterectomy, researchers administered a solution containing 200 mg of lidocaine with adrenaline (1: 500 000) or the same volume of saline in the pelvic cavity (control) (Narchi, et al. 1992). Lidocaine was absorbed from the abdomen in 5 minutes and reached a mean maximum concentration of 0.4 μg/ml at 3 hours. Results indicated that there were no differences between groups in scores for nausea, pain on movement, or morphine consumption, but pain scores at rest were significantly lower in the group that received lidocaine compared with the saline group (Williamson, Cotton and Smith 1997). However, in this study a low dose of lidocaine was administered, which could have negatively affected the results. This trial was

designed as a pilot for a study published a year later by the same research group (Ali, et al. 1998). In the later study, researchers increased the dose of lidocaine to 400 mg IP and surprisingly, lidocaine did not appear to provide analgesia after hysterectomy.

Contrasting results were obtained in studies that evaluated the analgesic properties of local analgesics administered IP after laparoscopic cholecystectomy, alone or in combination with other drugs (Elhakim, et al. 2000). A study from Thailand evaluated the analgesic effects of IP lidocaine alone or in combination with morphine for postpartum tubal ligation in a group of women that did not receive general anesthesia (Visalyaputra, et al. 1999). During the procedure, the patients were asked to express their pain in a scale of 1 to 10. Pain scores were significantly lower in patients that received IP lidocaine compared to patients that received morphine or saline. The dose of lidocaine in this study was 550 mg per patient and the highest mean plasma lidocaine level 2.7 μg/ml (range 1.3–3.7 μg/ml). These authors concluded that "IP lidocaine instillation, either alone or in combination with IM morphine, is a safe, effective, and easy technique that should be used to decrease suffering in patients who undergo postpartum tubal ligation under local anesthesia".

In a review article that questions the utility of IP administration of LAs for analgesia, authors analyzed the contrasting results published in different articles for reproductive surgery and laparoscopic cholecystectomy (Ng and Smith 2002). They concluded that IP administration of lidocaine was beneficial and that contradictory results between studies were due to differences in the type of surgery, dose, type and timing of IP instillation of LA and even a possible dilution factor in PF. A systematic review and meta-analysis evaluated the effect of IP local anesthesia in

laparoscopic cholecystectomy (Boddy, Mehta and Rhodes 2006). Sixteen randomized, controlled trials were analyzed for abdominal pain score. The principal outcome measure for quantitative analysis in this study was abdominal pain score at 4 hours after laparoscopic cholecystectomy; also additional analgesia requirements were compared. This review reported that IP instillation of LAs to reduce postoperative pain has been studied through randomized trials in human medicine for more than 10 years and that half of those studies reported a significant analgesic effect. No adverse events related to local anesthetic toxicity were reported in the studies analyzed. The authors concluded that IP administration of local anesthesia is safe, and it results in a statistically significant reduction in early postoperative abdominal pain.

Peritonitis in horses

Peritonitis, the inflammation of the thin membrane that lines the abdominal wall and covers most abdominal organs, can occur in association with a variety of disorders that result in mechanical, chemical or infectious insult to the peritoneal lining (Javsicas 2010). Regardless the cause, the initial immune response results in the release of histamine and serotonin from peritoneal mast cells and macrophages. These substances increase vascular permeability of the peritoneum leading to transudation of proteins and fluid. Macrophages also release chemotactic factors that increase neutrophil adhesion and migration. Then, peritoneal macrophages maintain the inflammatory response by production of platelet-aggregating factor, TNF-α, interleukin-1, prostaglandins, and leukotrienes. Recruitment and death of activated neutrophils produces

lysosomal degranulation and the release of degradative enzymes, such as superoxide and myeloperoxidase (Dabareiner 2009).

In a review of equine peritonitis, the most commonly described causes were duodenitis-proximal jejunitis, intestinal ischemia, intestinal perforation, rectal tears, intestinal parasites, abdominal neoplasia, hemorrhage, foaling/breeding trauma, umbilical infections, abdominal abscesses (often caused by *Streptococcus equi equi* or *Rhodococcus equi*), ruptured bladder, and neonatal septicemia (Davis 2003). But most authors agree that the most common causes of peritonitis in horses are secondary to acute GI diseases and abdominal surgery (Dukti and White 2008, Henderson, et al. 2008, Davis 2003). Iatrogenic peritonitis is also a common unintended complication of abdominocentesis during evaluation of horses with signs of abdominal pain. An experimental study found that after enterocentesis, PF had increased nucleated cell counts and specific gravity for at least 2 days (Schumacher, Spano and Moll 1985).

The clinical presentation of horses with peritonitis can vary depending on the cause, and some clinical signs can overlap with signs of the primary disease. Common systemic signs associated with peritonitis include colic, depression, tachycardia, pyrexia, decreased GI sounds, tachypnea, diarrhea, abnormal mucous membrane color, peripheral blood leukopenia or leukocytosis, and hyperfibrinogenemia (Hillyer and Wright 1997). Clinicopathologic signs of peritonitis are PF containing more than 10,000 nucleated cells/μl, total protein above 5 gr/dl (Henderson, et al. 2008), and PF cytology characteristics associated with inflammation, such as neutrophilia, evidence of cellular phagocytosis, presence of bacteria, karyolysis, or foreign material (Davis 2003).

Many inflammatory proteins and cytokines are associated with peritonitis in horses. The activity of TNF-α in particular, was reported to be increased in horses with acute abdominal disease (Barton and Collatos 1999). Based on previous evidence of the beneficial effect of IP administered lidocaine in the treatment of peritonitis in other species (Brocco, et al. 2008, Gallos, et al. 2004, MacGregor, Thorner and Wright 1980, Rimback, et al. 1988, Wilson, Barnes and Hauptman 2004, Yuzbasioglu, et al. 2008), it is possible that IP lidocaine could have a therapeutic effect in the treatment of horses with peritonitis. Furthermore, results from human research indicate that IP administration of lidocaine had beneficial effects on patients after abdominal surgery (Elhakim, et al. 2000, Klein 1990, Ng and Smith 2002, Visalyaputra, et al. 1999, Williamson, Cotton and Smith 1997) and thus could also be of value for horses that undergo abdominal surgery. The analgesic (Boddy, Mehta and Rhodes 2006) and anti-inflammatory properties of lidocaine (Brocco, et al. 2008, Rimback, et al. 1988, MacGregor, Thorner and Wright 1980) and even the potential of lidocaine to reduce abdominal adhesions (Yuzbasioglu, et al. 2008) are strong evidence that IP administration of lidocaine could improve the outcome of horses subjected to celiotomy for acute GI disease.

Use of peritoneal lavage for treatment of peritonitis

Treatment of horses with peritonitis depends on the cause of the inflammatory reaction. Common recommendations include IV fluid therapy, administration of broad spectrum antimicrobials, anti-inflammatory and anti-endotoxic drugs (Davis 2003). Abdominal lavage is

used in horses with the intention of removing blood, fibrin, inflammatory mediators, bile, pancreatic secretions, bacteria and foreign material from the abdomen and, in consequence, to reduce the intensity of peritonitis and the incidence of adhesion formation (Eggleston and Mueller 2003, Hague, et al. 1998). The abdomen can be lavaged during recumbent celiotomy or with the horse standing. Approximately 10-20 liters of an isotonic solution is often administered IP to bathe the entire abdominal cavity in a regular size horse (1100 lbs). An abdominal catheter can be placed during or after surgery for continued peritoneal lavage after recovery. Placement of abdominal catheters in standing horses is simple and associated with only minor complications, unless the bowel is perforated (Hague, et al. 1998). Standing horses usually are sedated to facilitate immobilization and to decrease the discomfort associated with the procedure. Ultrasound assistance can be employed to avoid the intestines and confirm the optimal location for placement of the catheter. A fenestrated large bore catheter is usually placed, such as a Foley catheter or those intended for thoracic drainage. An area of the ventral aspect of the abdomen, on midline, is aseptically prepared and then desensitized with subcutaneous administration of a local anesthetic. A small incision is made with a surgical blade through the skin and linea alba to allow the insertion of the catheter. If the catheter does not have a trocar to allow its insertion, forceps can be used to guide the catheter through the abdomen. It is important to perforate the peritoneum with the catheter to avoid instillation of fluid in the subcutaneous or subperitoneal tissues during lavage. The abdominal catheter can be secured to the skin with a purse-string suture or Chinese lock stitch, or if a Foley catheter is used insufflation of the balloon at the end of the catheter is often enough to keep it in place. To prevent ascending infection, it is

recommended to place a one-way valve (e.g. Heimlich valve) at the end of the catheter, which allows draining of the fluid from the abdomen while maintaining the vacuum in the abdomen (Davis 2003, Nieto, et al. 2003).

Minor complications associated with placement and maintenance of abdominal catheters were common (49%) in a retrospective study of 67 horses (Nieto, et al. 2003). The most commonly encountered problems were obstruction or slow drainage of fluid in 18 horses (26%), leakage of fluid around the drain in 11 horses (16%), and subcutaneous fluid accumulation around the drain in 8 horses (12%). These authors also mentioned the potential of the abdominal catheters to cause a sterile inflammatory reaction. Abdominal lavage in horses appeared to prevent adhesions in an experimental model (Hague, et al. 1998). Conversely, a questionnaire-based study found that the current use of intra-operative peritoneal lavage appeared not to be evidence-based in human medicine (Whiteside, et al. 2005).

Several substances have been added to abdominal lavage solutions in equine medicine, but in contrast with treatments of humans and other species, solutions containing LAs for abdominal lavage of horses have not been reported. Povidone iodine was tested in horses at different concentrations, but reports indicated that its infusion in the abdomen could cause chemical peritonitis (Schneider, et al. 1988). Moreover, studies in human models discourage the use of antiseptics -particularly povidone iodine- in abdominal lavage solutions (Qadan, et al. 2010). Researchers have proposed the addition of antibiotics and heparin to abdominal lavage solutions, but there is limited evidence to support their use in horses (Davis 2003) and some antibiotic drugs could irritate the peritoneum (Eggleston and Mueller 2003). However, a meta-

analysis study that evaluated the effect of peritoneal lavage on survival of rats and dogs with experimentally induced peritonitis concluded that there was a significant reduction in mortality when solutions that contained an antibiotic drug were administered (Qadan, et al. 2010).

Undesirable clinical signs associated with administration of lidocaine

Systemic Clinical Signs

Reported clinical signs of toxicity in horses range from skeletal muscle tremors to central neurological signs. In a study of standing horses an over-dose of lidocaine was administered intravenously until undesirable clinical signs were observed (Meyer, et al. 2001). The first clinical sign observed was skeletal muscle fasciculations, followed by behavior abnormalities that researchers described as "an alteration in visual function, rapid and intermittent eye blinking, anxiety and attempts to inspect closely located objects". Out of 19 horses in this study, one horse developed rapid onset of severe ataxia and collapsed into sternal recumbency moments after the onset of eye blinking, while seven horses appeared mildly sedated. The clinical signs reported in this study were mild when compared to signs of toxicity described in people, in which clinical signs associated with lidocaine toxicity are mainly localized to the central nerve system (CNS) and include dizziness, drowsiness, disorientation, respiratory depression, convulsions, coma, and death (Peat, et al. 1985).

Adverse Cardiovascular Effects

In people, lidocaine overdose has been reported to produce cardiac arrhythmias ranging from mild to life-challenging. Lidocaine can cause conduction delay that can vary from bundle branch block and prolonged P-R interval to complete heart block, sinus arrest, or asystole. Ventricular ectopy is common and could progress to more life challenging arrhythmias, such as ventricular tachycardia, torsades de pointes, and ventricular fibrillation (Weinberg 2002). In anesthetized dogs and in people, lidocaine overdose resulted in profound myocardial depression and hypotension (Groban, et al. 2001, Weinberg 2002). In contrast, no adverse cardiovascular effects were reported in studies of anesthetized horses that received IV lidocaine, even when plasma lidocaine concentrations were within the range in which other adverse reactions were observed (Enderle, Levionnois and Kuhn 2008, Doherty and Frazier, Effect of intravenous lidocaine on halothane minimum alveolar concentration in ponies 1998, Dzikiti, Hellebrekers and Dijk 2003, D. J. Feary, et al. 2005, Rezende, et al. 2011). In standing horses that received an overdose of lidocaine, statistically significant changes in P wave duration, P-R interval, R-R interval and Q-T interval were recorded when horses showed systemic signs of toxicity; however, these changes were considered clinically insignificant by the researchers (Meyer, et al. 2001). Furthermore, horses had heart rate, blood pressures and respiratory rates unchanged from control values.

Mechanisms of toxicity

Because of their chemical structure, LAs have lipophilic and hydrophilic properties that allow them to penetrate a variety of different cell membranes and organelles, and interact with cytosol and membrane-bound molecules. When LAs reach vital tissues (CNS, heart), they can interfere with cell signaling systems and energy transduction pathways, such as ionotropic signaling pathways (Na, potassium, and Ca ion channels), second messenger signaling systems (beta-adrenergic and lysophosphatidate), G-protein modulation of Ca and potassium channels, cardiac bioenergetics, and mitochondrial dynamics (adenosine triphosphate synthase). These mechanisms can subsequently alter cellular metabolism and homeostasis (Weinberg 2002). In neural tissue, for example, a study that investigated the neurotoxicity of lidocaine *in vitro* and *in vivo* showed that these effects were mediated by specific activation of the p38 mitogen-activated protein kinase in cells of the dorsal root ganglion, which is a potent activator of autodestructive pathways, such as the lipoxygenase system. However, it is likely that most of the neural clinical signs of toxicity can be attributed to block neuronal activity at the spinal dorsal horn level and subsequent modulation of the release of excitatory neurotransmitters (P. S. Tsai, et al. 1998). Specifically, it was determined that seizures induced by lidocaine in experimental conditions invariably start in the amygdala (De Toledo 2000).

Plasma Lidocaine Concentrations Associated with Toxicity

Although, adverse clinical signs associated with lidocaine overdose were observed at lidocaine plasma levels in the range of 1.85–4.53 μg/ml in standing horses (Meyer, et al. 2001),

other authors also described similar undesirable clinical signs when plasma concentrations of lidocaine were lower (Brianceau, et al. 2002, de Solis and McKenzie 2007, Milligan, et al. 2006) while other researches reported no signs of toxicity in horses with plasma lidocaine concentrations within 1.85–4.53 µg/ml (D. J. Feary, et al. 2005, Doherty and Frazier, Effect of intravenous lidocaine on halothane minimum alveolar concentration in ponies 1998, Valdeverde, et al. 2005). It is possible that many of the variables that affect the pharmacokinetics of lidocaine could alter the relationship between the dose administered and the plasma lidocaine concentrations obtained. Therefore, the presence of adverse clinical signs may not always correlate with the concentration of lidocaine in plasma. For example, in a study of human patients with arrhythmias, administration of lidocaine CRI produced unpredictable plasma lidocaine concentrations. These findings were attributed to variations in clearance of the drug, and apparent volume of distribution between patients with different cardiac diseases (Sawyer, Ludden and Crawford 1981). A forensic report described tissue concentrations (blood, liver, kidney, brain, lung, heart, vitreous humor, and urine) of lidocaine and MEGX in patients murdered by a nurse who injected overdoses of lidocaine (Peat, et al. 1985). Patients that were determined to have died of lidocaine overdose had hepatic lidocaine concentrations that ranged from 15 to 71 mg/kg, whereas patients that were receiving lidocaine infusions as an anti-arrhythmic treatment and died of other causes had lidocaine concentrations ranging from 0 to 14 mg/kg. Interestingly, MEGX was detected at relatively low concentrations in all of the murder cases in which it was examined.

Toxicity of MEGX and GX

Lidocaine may not be the only cause of undesirable clinical signs after its administration; in a study of prolonged administration of intravenous lidocaine in horses MEGX and GX were found to accumulate in tissues (de Solis and McKenzie 2007) and toxic effects of these metabolites were reported in other species (Blumer, Strong and Atkinson 1973). In rats, the median convulsant dose of lidocaine was 52 mg/kg and 67 mg/kg for MEGX. Conversely, convulsions were not observed even with lethal doses of GX, but it was proposed that GX could potentiate seizures when in combination with lidocaine and MEGX. These authors recommended that the combined concentrations plasma lidocaine and MEGX be determined to evaluate the risk of convulsions in patients treated with lidocaine; however, these maximum values have not been established in horses.

Treatment of lidocaine toxicity

In humans, lidocaine overdose can cause severe cardiac arrhythmia, profound myocardial depression and secondary hypotension, which could be life challenging. The focus of previous reviews on LAs overdose was prevention, early response, and treatment of hypotension and arrhythmias (Weinberg 2002), but recently remarkable resolution of toxicity was obtained by administering a lipid emulsion during resuscitation of people with LAs toxicity (Warren, et al. 2008). The exact mechanism of action of lipid infusion to treat local anesthetic toxicity has not yet been determined, but two main theories have been proposed (Weinberg, Lipid infusion

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therapy: translation to clinical practice 2008). In the lipid sink theory, it was theorized that lipophilic local anesthetic molecules partition into a lipemic plasma compartment making them unavailable to the tissue (Warren, et al. 2008). In contrast, a metabolic theory claims that LAs block the important oxidation of lipids in the cardiac muscle and the rapid infusion of lipids reactivates cardiac metabolism, therefore, the lipid infusion acts as a positive inotrope in the heart and reverses cardiac depression; this theory is supported by the positive effect of insulin-glucose therapy on bupivacaine cardiac toxicity (Weinberg, Lipid infusion therapy: translation to clinical practice 2008).

In contrast to other species, cardiovascular signs of clinical importance have not been reported in horses overdosed with lidocaine, and due to its short plasma life, most clinical signs of lidocaine overdose resolve within 10 to 40 minutes for most horses after discontinuation of the lidocaine infusion (Meyer, et al. 2001). Therefore, treatment of lidocaine toxicity may not be an important consideration in equine patients.

Chapter 2:
Pharmacokinetics of intraperitoneal infusion of lidocaine in horses

J. de Estrada, J.S. Taintor, J. Schumacher, D.M. Boothe

Objective – To describe the pharmacokinetics of intraperitoneal (IP) lidocaine in horses (30mg/kg).

Design – Double blinded cross-over, placebo controlled clinical trial, with a 2 weeks wash out period.

Setting – All horses were part of the research herd of the Auburn University Large Animal Teaching Hospital.

Animals – Four healthy adult, mixed breed horses, 8 to 15 years of age, that weighed between 490 and 570 kg were randomly assigned to receive either placebo or lidocaine first.

Interventions – A solution of 5 liters of balanced electrolyte solution with or without 2% lidocaine at a dose of 30 mg/kg was injected IP over 20 minutes. Horses were monitored for 24 hours after IP infusion for signs of toxicity. Blood was collected at 0, 5, 10, 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360, 480, and 1440 minutes after infusion. Samples of peritoneal fluid (PF) were obtained at minutes 0, 60, 120, 360 and 1440 minutes. Lidocaine and its active metabolite monoethylglycinexylidide (MEGX) were quantified in plasma and PF using high performance liquid chromatography. Time versus concentration data were subjected to non-compartmental analysis.

Measurements and Main Results – Peak plasma (C_{max}) lidocaine and MEGX concentrations were 2.82 ± 0.84µg/ml and 5.58 ± 3.78 µg/ml, respectively; time to maximum concentration was 75 ± 71 min and 93 ± 98 min respectively. Plasma lidocaine concentration remained above 1 µg/ml for 2 hours and declined to non-quantifiable concentration (< 0.2 µg/ml) by 8 hours after infusion. For IP, C_{max} for lidocaine and MEGX were 2.82 ± 0.84 and 5.58 ± 3.78 µg/ml, respectively. Clinical signs indicative of lidocaine adversity occurred in one horse after IP administration of lidocaine; this horse recovered completely in 45 minutes without intervention.

Conclusions – Mean plasma lidocaine concentrations were within target systemic levels (1-2 µg/ml) for 120 minutes after IP administration of 30 mg/kg, but signs of toxicity were observed in only one horse. Further studies are indicated to establish a IP dose of lidocaine necessary to achieve its target effects without causing adverse effects.

Key words: lidocaine, pharmacokinetics, intraperitoneal, horses

Introduction

Lidocaine, a local anesthetic, is beneficial for treatment of post-operative ileus (POI) in horses (Cook and Blikslager 2008, Malone, et al. 2006, Brianceau, et al. 2002, Torfs, et al. 2009). Lidocaine was considered a prokinetic drug by some authors (de Solis and McKenzie 2007, Torfs, et al. 2009, Koenig and Cote 2006), but studies failed to demonstrate a direct effect of lidocaine on the migrating myoelectric complex (Milligan, et al. 2006), gastric emptying, or cecal motility (Okamuraa, et al. 2009). Furthermore, continuous infusion of lidocaine increases transit time of feces in normal horses (Rusiecki, et al. 2008). Beneficial effects of lidocaine on

intestinal motility are more likely related to its anti-inflammatory properties (Cook and Blikslager 2008, Hollmann and Durieux 2000). Local anesthetics mitigate inflammation by their effects on granulocytes which include inhibiting the release of inflammatory mediators, lysosomal enzymes, and free radicals; neutrophil adhesion, migration, accumulation, and priming and nitric oxide generation; as well as inhibition of respiratory burst in macrophages (Hollmann and Durieux 2000). Therefore, lidocaine may prevent the deleterious effects of neutrophil migration to the intestinal wall (Cook and Blikslager 2008). Anti-inflammatory effects of lidocaine are most pronounced when lidocaine is applied topically rather than systemically possibly because higher tissue concentrations are achieved than can be attained via systemic administration (H. C. Caracas, J. V. Maciel, et al. 2009, Y. Azuma, et al. 2000).

In a recent publication (Peiro´, et al. 2010), intravenous administration of lidocaine was reported to have anti-inflammatory effects on horses with induced peritonitis. When delivered directly into the abdomen, anti-inflammatory and analgesic properties of local anesthetics have proven therapeutic value in treatment of peritonitis in rats (Brocco, et al. 2008, Yuzbasioglu, et al. 2008), rabbits (Rimback, et al. 1988), dogs (Wilson, Barnes and Hauptman 2004), and people (Ali, et al. 1998, Klein 1990, Narchi, et al. 1992, Ng and Smith 2002, Williamson, Cotton and Smith 1997, Chundrigar, et al. 1993, Carroll 2007, Elhakim, et al. 2000). Moreover, intraperitoneally administered lidocaine appears to be well-tolerated in people (Williamson, Cotton and Smith 1997, Narchi, et al. 1992, Ali, et al. 1998, Elhakim, et al. 2000, Visalyaputra, et al. 1999, Boddy, Mehta and Rhodes 2006), even when doses as high as 35 mg/kg are administered (Klein 1990).

In horses, intravenous infusion of lidocaine as a bolus of 1.3 mg/kg followed by continuous rate infusion (CRI) at 0.05 mg/kg/min. produced plasma concentrations that ranged from 1.21-3.13 µg/ml (Milligan, et al. 2006, D. J. Feary, et al. 2005, Brianceau, et al. 2002). The target plasma concentration for lidocaine for the treatment of POI was established at 1-2 µg/ml (Cook and Blikslager 2008). However, lidocaine has a narrow toxic range in horses and systemic administration for treatment of POI at commonly recommended doses produced markedly variable plasma lidocaine concentrations (Brianceau, et al. 2002). Horses administered an overdose of lidocaine exhibited undesirable clinical signs when the plasma lidocaine concentrations were in the range of 1.85–4.53 µg/ml (Meyer, et al. 2001). Remarkably, different studies reported adverse effects of intravenously administered lidocaine even in horses for which plasma concentrations of lidocaine are within or below the 1-2 µg/ml range (Brianceau, et al. 2002, de Solis and McKenzie 2007, Milligan, et al. 2006).

Considering that lidocaine anti-inflammatory properties were reported to be concentration-dependant (>1 mg/ml) (Y. Azuma, et al. 2000) but that high plasma lidocaine concentrations (1.85–4.53 µg/ml) (Meyer, et al. 2001) can cause signs of toxicity, we wondered if lidocaine could be administered intraperitoneally in horses in high concentration to achieve local therapeutic effects yet avoid clinical signs of toxicity. However, no study appears to have reported the pharmacokinetics of lidocaine administered IP to horses. The purpose of this study was to determine the pharmacokinetics of lidocaine and its major metabolite MEGX in healthy horses administered IP at a dose reported to have local (intraperitoneal) therapeutic effects (30 mg/kg) (Brocco, et al. 2008).

Methods

The study protocol was reviewed and approved by the Auburn University Institutional Animal Care and Use Committee (IACUC). Four healthy, mixed breed, adult horses, 8 to 15 years of age (mean age 12 years), 3 geldings and 1 mare that weighed between 490 and 570 kg (537.5 ± 33.8 kg) were studied. Horses were housed in a stall for 24 hours where they received their regular diet of coastal hay and pellets (12% protein) before and during the experiment.

Approximately twelve hours prior to study, each horse was restrained in stocks and an 18-G jugular vein, indwelling catheter[1] was placed for subsequent collection of blood samples. A 30-fr gold silicone-coated Foley catheter with a 30 ml blub[2] was aseptically placed in the abdomen for IP infusion, using a method previously described (Davis 2003). Discomfort related to placement of these catheters was minimized with sedation (5 mg of detomidine[3] IV), and local anesthesia (2% bupivacaine[4]).

Intraperitoneal infusion

Each horse was placed in a bedded stall and 5 liters of warm, balanced polyionic isotonic fluid[5], with or without a solution of 2% lidocaine HCl[6] was administered IP using an infusion set[7]. Infusion strength was calculated to deliver through the IP catheter 30 mg/kg lidocaine into the peritoneal cavity over 20 minutes. The IP catheter was occluded such that no fluid was allowed to drain. Twenty four hours after infusion, the catheter was removed and skin at the

catheter site was closed with staples[8]. Two weeks later the procedures were repeated using the alternate solution (i.e., saline with or without lidocaine).

Sample Collection

Blood samples (8 ml) were collected from the jugular catheter, into heparinized tubes[9] (Stargel, et al. 1979) before lidocaine was administered and at 5, 10, 15, 30, 45, 60, 75, 90, 120, and 150 minutes, and 3, 4, 5, 6, 8 and 24 hours following lidocaine administration. The jugular catheter was flushed with heparinized saline before and after each blood collection; 10 ml of blood were discarded before each sample was taken.

Peritoneal fluid was collected at minutes 0, and 60 minutes, 6 and 24 hours post infusion through an 18-ga., 3.8 cm needle inserted into the abdominal cavity near or on the ventral midline. Fluid was placed in a heparinized tube. Before each collection of peritoneal fluid, a clipped area of the right side of the abdomen, at least 15 cm away from the abdominal catheter was aseptically prepared by applying a five-minute scrub using chlorhexidine soap[10]. Samples were maintained below 4°C in heparinized tubes until cells were separated by centrifugation (1000 RCF for 10 minutes), then PF and plasma samples were stored frozen at -80 °C before analysis.

Determination of Lidocaine Concentrations

Plasma and peritoneal fluid samples were analyzed for concentrations of lidocaine and MEGX by high pressure liquid chromatography (HPLC) with ultraviolet (UV) detection, as described elsewhere (Milligan, et al. 2006, Doherty and Frazer 1998, C. Navas de Solis and

McKenzie 2007). The HPLC consisted of an autoinjector, binary pump, and Dual λ Absorbance detector (Waters Corporation™, Milford, MA, USA). Separation was achieved with a Kinetex C18, 3.6 µm, 150 x 4.6 mm column (Phenomenex®, Torrance, CA, USA). The mobile phase consisted of 85% 0.03M Potassium dihydrogen phosphate (VWR®, Radnor, PA, USA) and 15% acetonitrile (VWR®, Radnor, PA, USA) with the flow rate set to 1 ml/min. Lidocaine and MEGX were extracted from the samples with solid phase extraction (SPE) cartridges (Strata C8; Phenomenex, Torrance, CA, USA). Briefly, previously frozen samples were thawed and vortexed. The SPE cartridges were conditioned with 1 ml methanol and then 1 ml water. Sample (500 µl) was loaded followed by washing the cartridges with 500 µl 95% water: 5% methanol mixture. Finally, lidocaine and MEGX were eluted with 500 µl methanol which was then evaporated 25 min to dryness at 40 °C under a stream of nitrogen. The residue was reconstituted with 200 µl of mobile phase, and then the solution was centrifuged at 1900 x g. The injection volume was 150 µl. The retention time for lidocaine and MEGX were 7.0 min and 4.5 respectively and UV absorbance was monitored at 205 nm. Lidocaine and MEGX were quantitated in unknown samples based on signal comparison to a standard curve consisting of equine plasma samples that contained either lidocaine or MEGX at concentrations ranging from 100 to 5000 ng/ml (Sigma-Aldrich®, St. Louis, MO, USA) and MEGX (Grace®, Deerfield, IL, USA) reference standards and accepted if the coefficient of determination (r^2) was at least 0.999 and the predicted concentrations were within 10% of the actual concentrations. The linear correlation coefficient for lidocaine and MEGX was 0.999. The lower and upper limits of quantification for lidocaine and MEGX were 0.2 µg/ml and 5 µg/ml respectively. The relative

standard deviation (RSD) % for lidocaine 0.2, 0.5, 1, 2.5 and 5 μg/ml were 5.8%, 5.2%, 2.53%,

5.4% and 2.6% respectively. The RSD for MEGX 0.2, 0.5, 1, 2.5 and 5 μg/ml were 3.7%, 4.6%,

2.7%, 7.7%, 2.7%.

Pharmacokinetic Analysis

Plasma and peritoneal fluid concentration of lidocaine versus time data were subjected to

non-compartmental analysis (WinNonLin®). Plasma and peritoneal maximum concentrations

(C_{max}) and time to C_{max} (T_{max}) were obtained from recorded concentrations. Area under the time

curve (AUC_∞) (calculated to infinity using the trapezoidal method and loglinear rule), mean

residence time (MRT), and relative peritoneal bioavailability of lidocaine (AUC plasma/AUC

peritoneal) were also reported. Because lidocaine was not administered intravenously, the

constant of elimination, volume distribution, and clearance were not reported.

Evaluation of Systemic Effects

The study was designed as a double-blinded randomized (crossover) controlled clinical

trial. Two veterinarians, blinded to the solution administered, evaluated the systemic effects of

IP administration of lidocaine using scores for vital parameters, neurological, musculoskeletal

and cardiac examinations. Complete blood count, fibrinogen and serum biochemistries were

determined 12 hours before and 24 hours after IP infusion.

Arbitrary values were assigned to clinical parameters chosen to evaluate for undesirable

clinical signs that could be associated with lidocaine toxicity, such as attitude, presence of

nystagmus and other cranial nerve (CN) deficits, muscle fasciculations, ataxia, fecal consistency, (Gall and Roland Kaufmann 1996) (Table 1).

Table 1: Arbitrary scores assigned to each clinical sign to evaluate the systemic effects of IP administration of a solution containing 30 mg/kg of lidocaine or placebo to four healthy horses.

Attitude		Ataxia		Feces		Muscle Fasciculations		Nystagmus		CN Deficits	
Bright	1	No	0	Formed	1	No	1	No	1	No	1
Quiet	2	Minimal	1	Soft	2	Yes	2	Yes	2	Yes	2
Lethargic	3	Mild	2	Loose	3						
Disoriented	4	Marked	3	Diarrhea	4						
Depressed	5	May fall	4								
		Recumbent	5								

Evaluation of Local Effects

Local effects of lidocaine or placebo in the peritoneum were assessed by evaluating cell counts, total protein, specific gravity, color, and transparency of PF. Based on these findings, a pathologist (always the same), classified the PF as transudate, suppurative exudate, moderate suppurative inflammation, suppurative inflammation, or enterocentesis (Davis 2003).

Statistical analysis

Lidocaine and MEGX concentration data were reported as mean and standard deviation. A Mann-Whitney or paired T-test was used to compare plasma versus peritoneal Cmax for

lidocaine and MEGX. To detect changes in leukocyte counts and total protein in peritoneal fluid, the mean value on time 0 was compared with the mean leukocyte count on 6 and 24 hours by the use of repeated-measures analysis for a mixed generalized linear model. Temperature, heart rate, respiratory rate, peritoneal fluid leukocyte counts, total protein, and specific gravity were compared among the treatment groups by the use of a generalized linear model (paired T-test) with the Scheffe method used to adjust for multiple comparisons. For all analyses, values of $p < 0.05$ were considered significant.

Results

Plasma Lidocaine Concentrations

Mean peak plasma (C_{max}) lidocaine concentrations were 2.82 ± 0.84 μg/ml with a time to maximum concentration (T_{max}) of 75 ± 71 minutes, while C_{max} of MEGX was 5.58 ± 3.78 μg/ml at 93 ± 98 minutes. Peak plasma concentrations had a range of 1.82 - 4.07 μg/ml for lidocaine and 4.91 - 10.43 μg/ml for MEGX (Table 2). Lidocaine or MEGX were not detected in plasma or PF when the horses received the solution without lidocaine (control).

Table 2: Lidocaine and MEGX plasma and peritoneal average concentrations expressed as mean ± SD in μg/ml of 4 healthy horses that received an IP infusion of 30 mg/kg of lidocaine.

Time (min)	Plasma Lidocaine (μg/ml)	Plasma MEGX (μg/ml)	Peritoneal Lidocaine (μg/ml)	Peritoneal MEGX (μg/ml)
0	0	0	0	0
5	1.44 ± 0.7	2.08 ± 1.46		

10	2.03 ± 1.16	3.32 ± 2.62		
15	2.47 ± 1.34	4.27 ± 3.06		
30	2.53 ± 1.27	5.07 ± 3.57		
45	2.50 ± 1.21	5.27 ± 3.80		
60	2.05 ± 0.71	5.03 ± 3.31	981.48 ± 1515.10	12. 62 ± 5.30
75	1.74 ± 0.54	4.38 ± 2.7		
90	1.46 ± 0.40	3.79 ± 2.30		
120	1.26 ± 0.28	3.04 ± 1.76		
150	0. 93 ± 0.45	2.05 ± 1.07		
180	0.74 ± 0.73	1.36 ± 0.58		
240	0.52 ± 0.60	0.88 ± 0.27		
300	0.33 ± 0.47	0. 49 ± 0.069		
360	0.31 ± 0.45	0.34 ± 0. 094	166.15 ± 329	3 ± 3
480	0.15 ± 0.3	0. 07 ± 0.014		
1440	0	0	14.39 ± 21	0 ± 0

Average plasma lidocaine concentrations remained above 1μg/ml for 2 hours, declining to non-quantifiable (< 0.2 μg/ml) by 8 hours after infusion (Figure 1). The half-life in plasma was 69.51 ± 32 minutes for lidocaine and 84.81± 27 minutes for MEGX. The mean residence time (MRT) was 154.33 ± 147 minutes for lidocaine and 160.57 ± 111 minutes for MEGX. Plasma concentrations of MEGX paralleled the concentrations of lidocaine in each horse (Figure

1). For all horses lidocaine plasma C_{max} were above 1.85 µg/ml. The AUC_∞ was 393.32 ± 214

µg/ml for lidocaine and 726.42 ± 309 µg/ml for MEGX. The ratio of plasma lidocaine AUC

versus MEGX AUC was 0.54.

Figure 1: Semilogarithmic mean (± SD) lidocaine and MEGX plasma concentrations

(µg/ml) versus time (minutes) after IP administration of lidocaine at 30 mg/kg to 4

healthy horses.
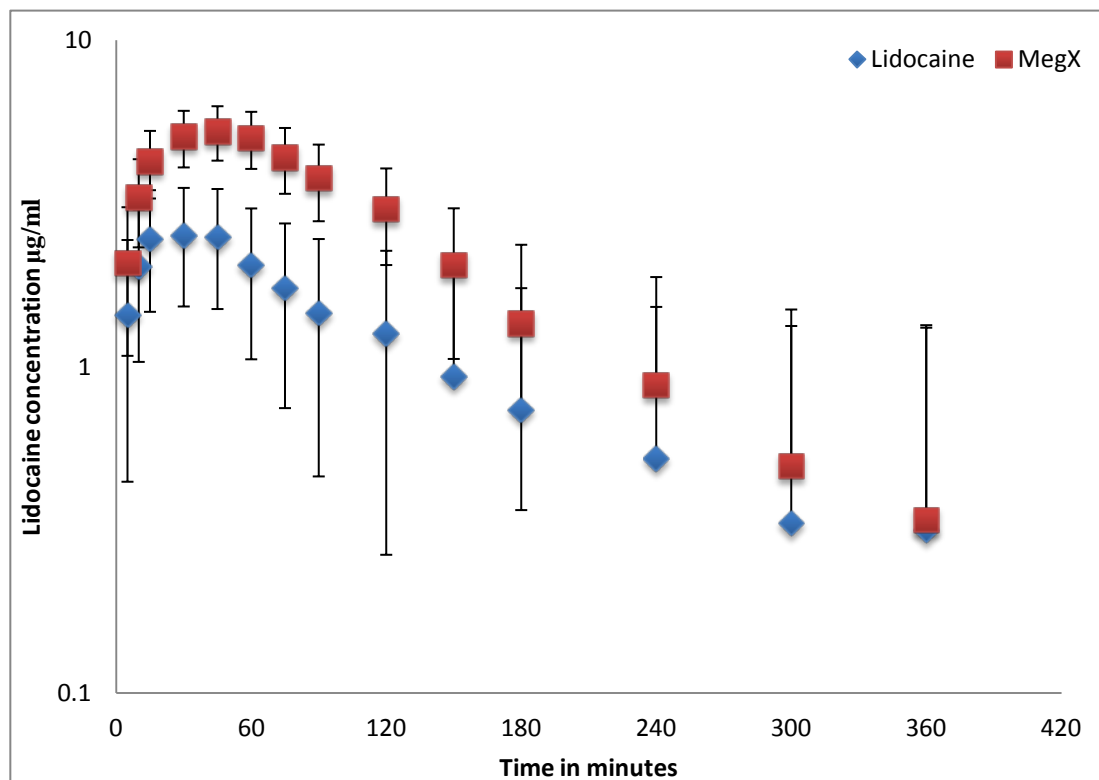
Peritoneal Fluid Lidocaine Concentrations

Lidocaine and MEGX concentrations in peritoneal fluid 60 minutes after IP infusion were 981.48 ± 1515 µg/ml and 12.62 ± 5 respectively. For each horse C_{max} of MEGX in the PF were higher than C_{max} of MEGX in plasma. The mean value of the ratio of MEGX in peritoneal to plasma concentrations was 2.37 ± 0.18 at minute 60 and then decreased to 1.04 ± 0.75 by minute 360. Concentrations of lidocaine ($p=0.02$) and MEGX ($p=0.03$) in peritoneal fluid were statistically higher than in plasma.

Figure 2: Semilogarithmic representation of the means of peritoneal lidocaine and MEGX concentrations (µg/ml) of 4 healthy horses that received 30 mg/kg of lidocaine
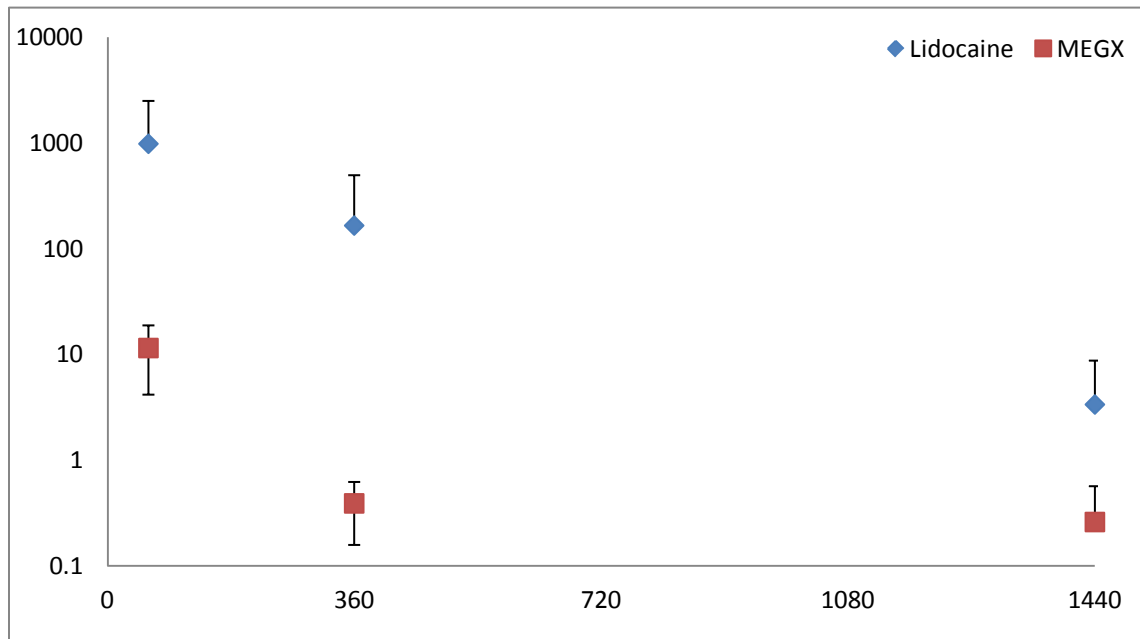
<u>Systemic Effects</u>

One horse (8-year-old gelding) developed undesirable clinical signs after IP administration of lidocaine. Fifteen minutes after infusion, he developed ataxia of grade 4/5, decreased limbs propioception, and cranial nerve deficits (VII and XII) (Reed and Andrews 2010). This horse had higher plasma concentrations of lidocaine (4.07 μg/ml) and MEGX (10.43 μg/ml) than other horses and concentrations peaked in this horse during the expression of clinical signs. All neurologic signs resolved within 45 minutes and did not return.

Another horse (10-years-old gelding) exhibited signs of abdominal pain (kicking at the abdomen, laying down, looking at the flank) between 60 and 120 minutes after IP infusion of both placebo and the solution with lidocaine. That the Foley catheter was correctly placed within the abdominal cavity was confirmed by ultrasonographic exam. This horse did not receive additional treatments and, because the signs of colic resolved after the intrabdominal catheter was removed, these signs were attributed to discomfort associated with the Foley catheter. The skin was closed with staples immediately after catheter removal to prevent loss of the solution administered. This horse also had hyperthermia (103.2°F) at 12 hours after infusion of the placebo solution, which resolved 12 hours later without medications. This horse did not develop clinically significant changes in the blood work. Remarkably, this horse had the lowest C_{max} of lidocaine (1.82 μg/ml), reaching a C_{max} later than other horses, at 180 minutes.

The rest of the horses had stable vital parameters throughout the experiment. CBC counts, fibrinogen and serum biochemistries for all horses were within reference limits for our laboratory for either treatment or before and after IP infusion for each individual. Subcutaneous

fluid accumulation around the drain was observed in most horses approximately 12 hours after IP infusion. This minor complication was also reported in 12% of 67 horses that had intra-abdominal drains placed during abdominal surgery (Nieto, et al. 2003) and resolved by 24 hours after IP infusion in our horses.

Local Effects

All horses developed peritonitis (defined as a nucleated cell count >10,000 cells/µL) (Davis 2003), which was detected in the sample of PF obtained immediately before infusion of the IP solution, but resolved by the end of the 2 week washout period. The peritoneal reaction to the IP catheter ranged from mild to severe suppurative inflammation. The average of nucleated cells in the PF 12 hours after placement of abdominal catheters was 164,393 leukocytes/µl (Figure 4) which were mostly neutrophils. No infectious organism or sign of infection were observed in cytological evaluation of the PF, but the fluid was not cultured. Collection of peritoneal fluid was complicated by accidental enterocentesis on 2 occasions in the same horse, at time 0, during both trials (1 in with the control solution and the other one with lidocaine; cytological results excluded from the study.) which could have affected the results of subsequent PF analyses (Schumacher, Spano and Moll 1985). Statistical differences could not be detected between groups in the number of white blood cells in the peritoneal fluid. However, total protein in the peritoneal fluid was decreased in all horses at 60 minutes when compared to time 0 and 24 hours (p<0.03), but was not different between treatments. Despite the increased concentration of leukocytes, the pH of the peritoneal fluid was alkaline before (9.1 ± 0.54) and after IP infusion

(8.5 ± 1.4). Statistical differences could not be detected for peritoneal fluid pH among times (0, 60, 360, and 1440) or treatments (lidocaine versus placebo). All horses remained apparently healthy after the experiment was finished.

Figure 3: Representation of the total nucleated cells in the peritoneal fluid of 4 healthy horses 12 hours after placement of an abdominal catheter. At time 0 all horses received an intraperitoneal infusion of 30 mg/kg of lidocaine or placebo.
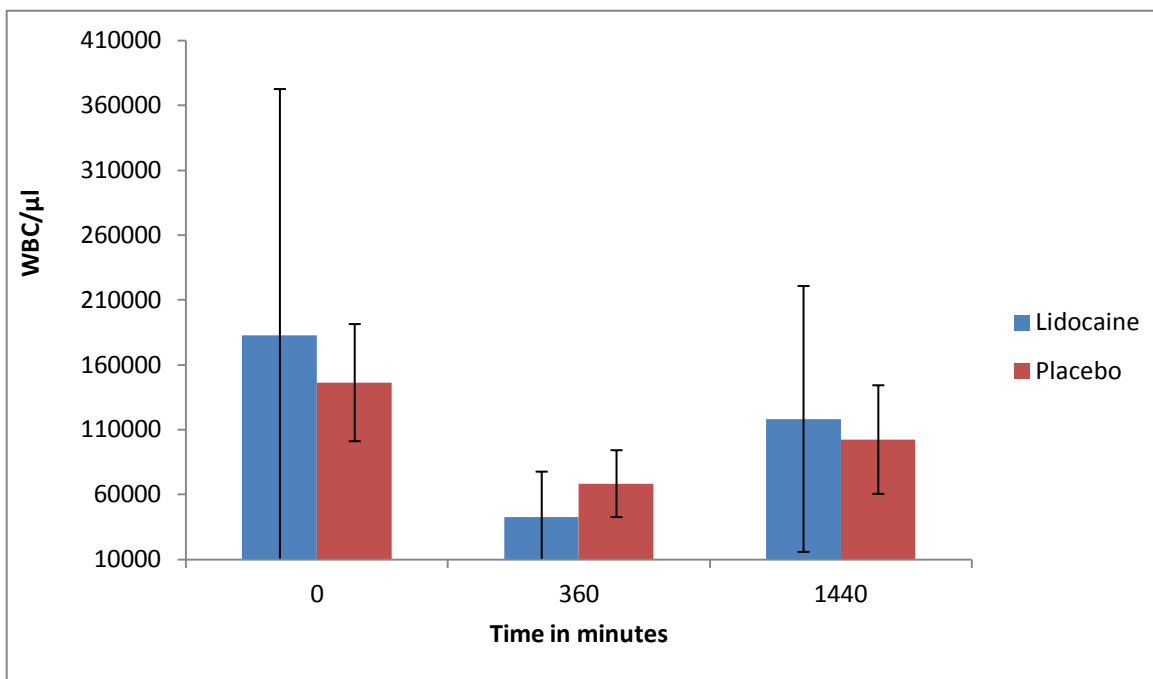
Discussion

The information collected in this study documents the pharmacokinetics of an IP injection of lidocaine at 30 mg/kg. This dose was 23 times higher than the dose commonly used

for a loading dose of lidocaine (1.3 mg/kg) for treatment of horses with POI (Cook and

Blikslager 2008).

Intraperitoneal administration of lidocaine at 30 mg/kg produced mean plasma C_{max} of

2.88 ± 0.95 µg/ml. This concentration is comparable to C_{max} reported by others after systemic

administration. Studies that administered a loading dose of 1.3 mg/kg IV followed by a CRI of

0.05 mg/kg/min IV to conscious horses reported mean C_{max} of 2.0 ± 0.27 µg/ml (D. J. Feary, et

al. 2005), and 2.61 ± 0.38 µg/ml (Milligan, et al. 2006). Further, the mean Cmax obtained in our

study is even lower than the C_{max} reported after IV administration of a loading dose of 1.3 mg/kg

IV followed by a CRI of 0.05 mg/kg/min IV to horses receiving general anesthesia (3.8 ± 0.55

µg/ml) (D. J. Feary, et al. 2005).

Although adverse clinical signs associated with lidocaine toxicity were reported in horses

that had plasma concentrations of lidocaine in the range of 1.85–4.53 µg/ml (Meyer, et al. 2001),

other authors have described similar undesirable clinical signs at lower plasma concentrations

(Brianceau, et al. 2002, de Solis and McKenzie 2007, Milligan, et al. 2006). However, no signs

of toxicity were reported in horses for which plasma lidocaine concentrations exceeded 1.85

µg/ml (D. J. Feary, et al. 2005, Valdeverde, et al. 2005, Doherty and Frazer 1998). In our study,

plasma lidocaine reached or exceeded 1.85 µg/ml, yet, only one horse exhibited signs of toxicity.

These discrepancies in concentration versus toxicity response relationships may reflect the lack

of correlation between concentration and adversity. Perhaps equally problematic is the role of

MEGX and GX in causing adversity. One study has demonstrated increasing plasma

concentrations of MEGX and GX after prolonged administration of IV lidocaine (de Solis and

McKenzie 2007). Toxic responses to these metabolites have been observed in other species (Blumer, Strong and Atkinson 1973).

Plasma and peritoneal lidocaine levels were variable between horses. This could be interpreted as differences in the absorption or elimination of the drug from the abdomen for each horse, as it is reflected in the high standard deviation obtained. High variability in plasma lidocaine concentration after its administration is not an uncommon finding as it was reported in other studies (de Solis and McKenzie 2007, Milligan, et al. 2006, Brianceau, et al. 2002, Valdeverde, et al. 2005). Pharmacokinetics of lidocaine can be affected by many variables, such as renal function, fasting (Engelking, et al. 1987), acid base status (Yakatis, Thomas and Mahaffey 1976), albumin binding capacity (Milligan, et al. 2006), liver function (Orlando, et al. 2004), cardiac output (Collinsworth, Kalman and Harrison 1974), and endotoxic shock (McKindley, et al. 2002, Peiro´, et al. 2010).

Following systemic administration, lidocaine has a high hepatic extraction rate, as it is metabolized in the liver via oxidative N-dealkylation by the cytochrome P450 system (CYP) to MEGX (Alexson, et al. 2002). In humans, CYP-1A2 is a major determinant of lidocaine metabolism *in vivo* (Orlando, et al. 2004), whereas CYP-2B1 and 3A2 where the major P450 isoforms in rats (Nakamoto, et al. 1996). MEGX is then further metabolized to glycinexylidide (GX) and other metabolites that are later excreted with the bile and urine (Collinsworth, Kalman and Harrison 1974).

In contrast to our results, other studies reported MEGX plasma concentrations lower than lidocaine plasma levels (Valdeverde, et al. 2005, Robertson, et al. 2005). In our study, plasma

MEGX levels were higher than lidocaine plasma concentrations. This may reflect direct absorption of lidocaine from the peritoneum into portal circulation and rapid hepatic metabolism of the drug (Alexson, et al. 2002). Because the amount of intraperitoneal lidocaine in our horses was 23 times higher than the loading dose used for IV administration, the mean MEGX plasma levels in our samples were much higher than those levels reported by other authors (Valdeverde, et al. 2005, de Solis and McKenzie 2007, Robertson, et al. 2005).

A limitation of our study was that lidocaine was not administered IV; therefore, the rate constant of elimination was not determined and the volume of distribution and clearance were not obtained. Further, absolute bioavailability could not be determined for IP administration. In a study with conscious horses (n=8) that received a dose of lidocaine of 1.3 mg/kg over 15 minutes followed by a CRI of 0.05 mg/kg/min, lidocaine Cl was 29 ± 7.6 ml/min/kg, VD at steady state was 0.79 ± 0.16 L/kg. (D. J. Feary, et al. 2005).

Our results indicated a half-life of lidocaine in plasma of 69.51 ± 32 minutes and 84.81 ± 27.41 minutes for MEGX. The mean residence time (MRT) was 154.33 ± 147 minutes for lidocaine and 160.57 ± 111 minutes for MEGX. In conscious horses the terminal half-life of lidocaine was 79 ± 41 minutes, but the MRT was much shorter, at 28 ± 7.8 minutes (D. J. Feary, et al. 2005). In a group of horses in post-operatory recovery, the elimination half-life after discontinuation of lidocaine infusion was 48.01 ± 22.71 minutes for lidocaine, 123.96 ± 71.02 minutes for MEGX (de Solis and McKenzie 2007), but horses under general anesthesia had higher lidocaine C_{max}, smaller volume of distribution, lower clearance, and shorter half-life (Table 3) (D. J. Feary, et al. 2005). Our results differ from other studies, probably because

lidocaine was absorbed from the peritoneal cavity into the portal circulation before it reached systemic concentrations. However, the half-life of lidocaine was shorter than the half-life of MEGX, as previously reported in horses that received systemic administration of lidocaine (de Solis and McKenzie 2007).

The lidocaine AUC_∞ of our study (393 ± 214 µg.min/ml) was higher than the value reported in conscious horses (210 ± 52 µg/ml), but similar to the value reported in anesthetized horses (410 ± 84 µg/ml) that received a dose of lidocaine of 1.3 mg/kg over 15 minutes followed by a CRI of 0.05 mg/kg/min (D. J. Feary, et al. 2005). The AUC_∞ for MEGX was 726 ± 310 µg.min/ml, which represents approximately an 80% more MEGX in plasma than lidocaine (Table 3). This could be due to the rapid metabolism of lidocaine to MEGX and possibly a slower renal excretion of MEGX in relation to lidocaine (de Solis and McKenzie 2007).

Table 3: Mean $\pm$ SD for kinetic values of plasma lidocaine and MEGX after lidocaine IP at 30 mg/kg to 4 horses compared to values of conscious horses that received a bolus of 1.3 mg/kg and a CRI of 0.05 mg/kg/min IV in a different study (D. J. Feary, et al 2005)

Protocol	30 mg/kg IP over 20 minutes	1.3 mg/kg and a CRI of 0.05 mg/kg/min IV	
Kinetic Value	Lidocaine	Lidocaine Awake	Lidocaine Anesthetized
C_{max} (µg/ml)	2.88 ± 0.95	2.0 ± 0.27	3.8 ± 0.55
T_{max} (min)	71.25 ± 74.87	22 ± 28	23 ± 21
$T_{1/2}$ (min)	69.51 ± 32	79 ± 41	54 ± 14
MRT (min)	154.33 ± 147	28 ± 7.8	27 ± 5.4
AUC_∞ µg/ml	393.32 ± 214.22	210 ± 52	410 ± 84
C_{max} = Maximum plasma drug concentration; T_{max} = Time until C_{max}; $T_{1/2}$: Terminal half life; MRT: Mean residence time; AUC_∞: Area under the curve extrapolated to infinity			

Statistical differences could not be detected between treatments for the clinical

parameters, and white blood cells in the abdomen. Other studies have reported that migration of

equine leukocytes is not affected by lidocaine, which is in contrast to other species (Cook, et al.

2009, Peiro´, et al. 2010). One of the limitations of this study is that we did not characterize

inflammatory or other characteristics of peritoneal fluid (lactate, glucose) indicative of peritoneal

response to lidocaine. Total protein in the peritoneal fluid was statistically lower in both groups

at 60 minutes when compared to time 0 and 24 hours, but not different between treatments. This

could be explained by a dilution factor after intraperitoneal administration of the solution and

may not be clinically significant. It is likely that the clinicopathologic signs of aseptic peritonitis

observed in all peritoneal samples 12 hours after placement of the abdominal catheter were

caused by a reaction to the Foley catheter. Sterile technique was used for the placement of intra-

abdominal catheters and samples of abdominal fluid were obtained from areas of the abdomen at

least 15 cm from the catheter.

Blood work values and physical examinations performed at the beginning and at the end

of the study confirmed the healthy status of the horses of this study. Differences in CBC counts,

fibrinogen or serum biochemistries were not detected between groups. Induction of aseptic

peritonitis by placement of intra-abdominal catheters has not been previously used as a model of

peritonitis, but it caused significant changes in the characteristics of the peritoneal fluid in every

one of the horses in our study. It is possible that peritonitis could have affected the

pharmacokinetics of lidocaine after peritoneal administration. However, peritonitis is also

common after abdominal surgery (Mair and Smith 2005); therefore, our study may more closely

mimic real-life variables for horses with intrabdominal catheters or with aseptic peritonitis post-abdominal surgery.

Interestingly, the horse that had the highest plasma lidocaine and MEGX concentrations was the same horse that had the enterocentesis performed at time 0. It is possible that the inflammation caused by the enterocentesis increased the absorption of lidocaine from the abdomen, which could be reflected in the higher plasma lidocaine and MEGX concentrations compared to the rest of the horses. It was surprising that the pH of the peritoneal fluid in horses of this study was alkaline which is in contrast to a study (Hoogmoed, et al. 1999) that reported a pH below 7.3 in PF of horses with peritonitis. The alkalinity of the pH in the peritoneal fluid could have increased the absorption of lidocaine from the abdomen. At a pH of 9, for example, 82% of the lidocaine would be unionized and thus more able to cross membranes.

Even though a lidocaine concentration of 981.48 ± 1515 μg/ml was detected in the peritoneal fluid at time 60, mean plasma lidocaine concentrations were comparable to those reported by other authors using a dose of 1.3 mg/kg over 15 minutes followed by a CRI of 0.05 mg/kg/min in another study (Table 3) (D. J. Feary, et al. 2005). In other species, IP administration produced plasma lidocaine concentrations similar or lower than those achieved with systemic administration (Labaille, et al. 2002, Narchi, et al. 1992, Wilson, Barnes and Hauptman 2004, Williamson, Cotton and Smith 1997)

It was remarkable that peritoneal MEGX concentrations were higher than the plasma MEGX levels, as it was reflected in the ratio of MEGX in PF to plasma (2.37 ± 0.18 at minute 60). It is possible that peritoneal fluid concentrations of lidocaine and MEGX could be higher

than plasma after IV administration as well, but we could not find a study that reported this finding. It is possible that MEGX bound to peritoneal proteins and was then released slowly from the abdomen. But in our horses the estimated total protein (based on specific gravity) of the peritoneal fluid was within the reference range for normal horses (2.37 ± 1.3 mg/dl), (Davis 2003). Another possible explanation would be that the higher concentration of MEGX in the peritoneal cavity compared to plasma concentrations could be due to extrahepatic metabolism of lidocaine (Sallie, Tredger and Williams 1992). In a rat model, it was estimated that approximately 30% of the metabolism of lidocaine to MEGX was extrahepatic (Ping, et al. 2001), but the amount of lidocaine that undergoes extrahepatic metabolism in the horse has not been reported. White blood cells in the peritoneal fluid could have metabolized lidocaine into MEGX, leading to the high concentrations of MEGX in the peritoneal fluid, but we did not find reports that documented the metabolism of lidocaine by leukocytes or mesothelial cells from the peritoneum. Finally, it is possible that some lidocaine could have degraded in the peritoneal fluid. However, lidocaine is resistant to hydrolysis, even in acidic or basic pH, and when it degrades, lidocaine is transformed into 2,6-dimethylaniline and N, N-diethylaminoacetic acid (Powell 1987), instead of to MEGX.

The results of our study showed that IP administration of lidocaine at 30 mg/kg can be administered to horses to provide high concentrations of lidocaine in the peritoneal cavity (981.48 ± 1515 μg/ml). Lidocaine plasma concentrations were comparable to those reported in other studies with horses (D. J. Feary, et al. 2005, D. Feary, et al. 2006, Milligan, et al. 2006, Rezende, et al. 2011, Brianceau, et al. 2002). Lidocaine's main metabolite, MEGX accumulated

in the PF and reached systemic concentrations higher than lidocaine. This is the first report of IP administration of lidocaine to horses, an innovative method of administration. Further research, however, is needed to determine its potential therapeutic use in horses with peritonitis.

Manufacturer's details

1. Abbott Laboratories. Abbott Park, Illinois, USA

2. KenGuard, The Tendal Company, Mansfield, MA, USA

3. Dormosedan, Pfizer, Inc., Exton, PA, USA

4. Bupivacaine hydrochloride injectable-0.5%: Hospira, Inc., Lake Forest IL, USA

5. Veterinary Plasma-lyte A: Abbott Laboratories, North Chicago, IL, USA

6. Lidocaine hydrochloride injectable-2%, VEDCO, INC., St. Joseph, MO, USA

7. Rapid Infusion Transfer Set, Mila International Inc., Erlanger, KY, USA

8. Weck Visistat 35W, Teleflex Medical, Research Triangle Park, NC, USA

9. Monoject, Tyco Healthcare Group LP, Mansfield , MA, USA

10. Vet One, MWI Meridian, ID, USA

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