The Veterinary Journal 196 (2013) 461–466

Contents lists available at SciVerse ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Lidocaine and structure-related mexiletine induce similar contractility-enhancing effects in ischaemia–reperfusion injured equine intestinal smooth muscle in vitro

Karen Tappenbeck [a], Susanne Hoppe [a], Klaus Hopster [b], Manfred Kietzmann [c], Karsten Feige [b], Korinna Huber [a,*]

[a] Department of Physiology, University of Veterinary Medicine, Bischofsholer Damm 15, D-30173 Hannover, Germany
[b] Clinic for Horses, University of Veterinary Medicine, Bünteweg 9, D-30559 Hannover, Germany
[c] Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany

ARTICLE INFO

Article history:
Accepted 15 November 2012

Keywords:
Lidocaine
Prokinetic
Ischaemia–reperfusion
Intestinal
Smooth muscle

ABSTRACT

Postoperative ileus (POI) is a severe complication following small intestinal surgery in horses. It was hypothesised that prokinetic effects of lidocaine, the most commonly chosen drug for treatment of POI, resulted from drug integration into smooth muscle (SM) cell membranes, thereby modulating cell membrane properties. This would probably depend on the structural and lipophilic characteristics of lidocaine. To assess the influence of molecular structure and lipophilicity on prokinetic effects in vitro, the current study compared the effects of lidocaine with four structure-related drugs, namely, mexiletine, bupivacaine, tetracaine and procaine.

The response to cumulative drug administration and reversibility of effects were tested by measuring isometric contractile performance of equine jejunal circular SM strips, challenged by a standardised, artificial in vivo ischaemia–reperfusion injury. A second set of SM strips were incubated with the different drugs to determine changes in creatine kinase (CK) release. All drugs caused a drug-specific increase in contractility, although only lidocaine and mexiletine induced similar concentration-dependent curve progressions, significantly reduced CK release, and featured shorter recovery times of tissue contractility after washing, compared to bupivacaine and tetracaine. In was concluded that the structural and lipophilic similarity of mexiletine and lidocaine were responsible for the similar effects of these drugs on SM contractility and cell membrane permeability, which supported the hypothesis that prokinetic effects of lidocaine are based on interactions with SM cell membranes modulated by these features.

Introduction

Postoperative ileus (POI) occurs as a severe complication following small intestinal surgery in horses (Cohen et al., 2004). A study by Van Hoogmoed et al. (2004) revealed that lidocaine, a local anaesthetic and class 1B antiarrhythmic agent, is the most common drug used to treat POI. Various studies have reported beneficial effects of therapeutic or prophylactic lidocaine treatment of horses with POI or likely to develop POI after gastrointestinal surgery (Cohen et al., 2004; Malone et al., 2006; Torfs et al., 2009). In contrast, gastrointestinal motility of normal, non-injured gut could not be enforced by lidocaine administration (Milligan et al., 2007). Recent in vitro studies have confirmed the direct contractility-enhancing effects of lidocaine on equine jejunal smooth muscle (SM), which were more pronounced in injured than in control tissues (Guschlbauer et al., 2010a). Nevertheless, cellular mechanisms underlying these effects remain unclear.

In addition to contractility-enhancing effects, several studies have shown the beneficial effects of lidocaine on creatine kinase (CK) release from cardiac and intestinal muscle challenged by ischaemia and reperfusion, indicating membrane protecting effects (Takeo et al., 1989; Guschlbauer et al., 2010a). Further evidence of membrane interactions of lidocaine with various cell types has been provided by studies reporting that lidocaine induced dose-dependent shape change in human erythrocytes by modulating the structural properties of the cell membrane skeleton (Nishiguchi et al., 1993, 1995) and caused disruption of erythrocyte membrane microdomains (Kamata et al., 2008), known as lipid rafts.

It is therefore feasible that both contractility-enhancing and cell membrane protecting effects of lidocaine resulted from drug integration into SM cell membranes, modulating cell membrane properties. For these effects, structural and lipophilic characteristics of lidocaine would play a major role. To assess the influence of molecular structure and lipophilicity on contractility-enhancing and cell membrane protecting effects, the current study compared the effects of lidocaine to those of four structure-related drugs, namely, mexiletine, bupivacaine, tetracaine and procaine. A model of

* Corresponding author. Tel.: +49 511 856 7543.
E-mail address: Korinna.Huber@tiho-hannover.de (K. Huber).

1090-0233/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tvjl.2012.11.011

equine jejunal SM, challenged by artificial in vivo ischaemia–reperfusion, was used since lidocaine was shown to be more effective at modulating injured SM.

Materials and methods

Surgical procedure and ischaemia–reperfusion injury

In this study nine mature Warmblood mares of different breeds and one Thoroughbred mare were used. All horses were 14.2 ± 1.0 (mean ± SEM) years old, dewormed and did not show any signs of gastrointestinal disorders. Horses were kept in individual stalls with free access to water and were fed hay and concentrated feed three times a day for at least 5 days before surgery.

For sampling, a standardised, artificial in vivo ischaemia–reperfusion model was used, as previously described (Guschlbauer et al., 2010a). Prior to surgery, horses were fasted for 6 h, then premedicated with xylazine (0.8–1.1 mg/kg bodyweight [BW] IV) before induction of anaesthesia with diazepam (0.05 mg/kg BW IV) and ketamine (2.2 mg/kg BW IV). Balanced anaesthesia was maintained with isoflurane in 100% oxygen and continuous rate infusion of xylazine at 0.7 mg/kg BW/h. Dobutamine, lactated Ringer's solution and hydroxyethyl starch (hetastarch) were administered to maintain a mean arterial blood pressure above 60 mmHg during anaesthesia.

During a routine median laparotomy, an artificial ischaemia–reperfusion injury was induced on a 25 cm segment of distal jejunum located 1.5 m orally of the ileocaecal fold. Penrose drains were used to ligate the mesenteric blood vessels (but maintain blood vessel integrity) and the lumen of the intestinal segment. Ringer's solution at body temperature was used to distend the intestinal wall to an intraluminal pressure of 20 cm H_2O. During the 15 min of ischaemia the distended intestine was replaced into the abdominal cavity.

Following ischaemia, all Penrose drains were removed to reinstate circulation and the intraluminal fluid was emptied into the caecum. After replacing the intestine into the abdominal cavity for 15 min of reperfusion, the ischaemia–reperfusion injured intestinal segment was resected and the horse was euthanised without regaining consciousness using pentobarbital (35 mg/kg BW IV). All procedures were approved by the State Office for Consumer Protection and Food Safety in accordance with the German Animal Welfare Law (Application Number 33.9-42502-04-10/0241).

Tissue preparation and drug administration

The resected intestinal segment was transferred into modified Krebs–Henseleit Buffer (KHB; in mmol/L: 117.0 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 11.0 glucose, 25.0 $NaHCO_3$; 38 °C; pH 7.4, continuously aerated with 95% O_2 and 5% CO_2) and prepared as previously described (Guschlbauer et al., 2010a). Mucosa and submucosa were removed and circular SM strips of equal size and weight were prepared (0.5 × 1.0 cm; 0.029 ± 0.001 g wet tissue).

For measuring isometric contractile forces SM strips were placed into organ baths filled with 12 mL KHB and connected to isometric force transducers (HBM). Initial tension was adjusted at 2 g in the light of preliminary studies in which 2 g of initial tension resulted in optimal SM length for maximum isometric force development (Nieto et al., 2000). After 35 min of equilibration, viability of the enteric nervous system (ENS) and SM cells was documented by contractile response to electric field stimulation (10 s, 10 Hz, 30 V).

To ensure that measured responses to drug administration were only due to contractile activity of the SM and not to activation of the ENS, the ENS was deactivated using 1 μmol/L tetrodotoxin (Biotrend Chemicals) (Boddy et al., 2004). Successful inhibition of the ENS was documented by lack of contractile response to electric field stimulation 15 min after administering tetrodotoxin. Lidocaine, bupivacaine, mexiletine, procaine and tetracaine in form of their hydrochlorides (Sigma–Aldrich) were dissolved in distilled water and added cumulatively to the organ baths, starting 30 min after application of tetrodotoxin. Drugs were applied to the organ baths in volumes of 0.72–120 μL at intervals of 10 min, resulting in drug concentrations in the organ baths of 0.004, 0.009, 0.019, 0.037, 0.074, 0.185, 0.369, 0.739 and 1.477 mmol/L. As control values, the isometric contractile performance of ischaemia–reperfusion injured SM strips without local anaesthetic treatment was recorded simultaneously. Values were received from two (lidocaine, untreated) or three (mexiletine, bupivacaine, tetracaine, procaine) SM strips/horse. For measuring SM contractile activity a Spider8 chart recorder (4.8 kHz/DC; HBM) was used in combination with Catman Easy software (version 1.01; HBM).

Contractility

Contractility of SM was defined as isometric contractile performance of SM strips, described by mean active force (*MAF* [mN]), frequency (*F* [peaks/min]) and amplitude (*A* [mN]) of contractions.

Mean active force, frequency and amplitude of contractions

After each drug administration SM strips were given a 5 min equilibration time. *MAF*, *F* and *A* were analysed during 5 min evaluation periods starting 5 min after drug administration until directly before the next drug administration. For calculating *MAF*, baseline values were subtracted from the arithmetic mean of all values describing the curve during the corresponding evaluation period. For statistical analysis of *F* and *A* all peaks during the evaluation periods were registered. Peaks were defined as contractions with amplitudes of at least 0.2 g (1.96 mN).

Recovery times after washing

Reversibility of drug effects was tested by washing the SM strips twice with KHB after the final drug administration at intervals of 10 min. To determine recovery times after washing, *MAF* values were calculated for the last minute before the first wash. After washing, recovery of contractility was specified as increase in *MAF* to values of at least 150% of values directly before the first wash and of at least 0.98 mN. Time periods between the first wash and recovery of contractility after washing were recorded and defined as recovery times.

Creatine kinase release from smooth muscle cells

A second set of SM strips (2.0 × 2.0 cm; 0.3075 ± 0.016 g wet tissue) was prepared and incubated for 5 min in 5 mL KHB containing 0.185 mmol/L lidocaine, mexiletine, bupivacaine, tetracaine or procaine. As control samples, SM strips were incubated in KHB without drug supplementation. After incubation CK activity in the incubation buffer was measured using an automated analyser (Vitros DT60 II, Ortho-Clinical Diagnostics).

Statistical analyses

Statistical analyses were performed using GraphPad Prism 4.0 (GraphPad Software) for Microsoft. All values were expressed as means ± SEM of 10 horses. Each mean was composed of two (lidocaine, untreated) or three (mexiletine, bupivacaine, tetracaine, procaine) values/horse. Significance was set at $P < 0.05$ with $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. $P < 0.1$ was defined as a trend. *MAF*, *F* and *A* of lidocaine treated tissues were compared to values of untreated tissues using Paired *t* tests for each drug concentration. For the concentration range in which lidocaine induced a significant increase in contractility two-way repeated measures ANOVA (factors concentration and treatment) were performed. Effects of the different drug treatments were analysed by one-way repeated measures ANOVA with Tukey's post hoc test regarding the influence on *F*, *A* and *MAF* at each drug concentration, comparing mexiletine, bupivacaine, tetracaine or procaine treated tissues to lidocaine treated tissues, respectively. Due to technical reasons recovery times after washing and CK activity in the incubation buffer were only measured in nine horses. For statistical analysis of recovery times after washing one-way repeated measures ANOVA with Tukey's post hoc test was performed. CK activity in the incubation buffer was analysed using Paired *t* tests, comparing each drug treatment to untreated control tissues.

Results

Mean active force, frequency and amplitude of contractions

In the concentration range of 0.074–0.739 mmol/L lidocaine induced a significant, concentration-dependent increase in *MAF* compared to untreated control tissues (Fig. 1a). This increase was mainly based on a rise in *F* (Fig. 1b), whereas the effect on *A* was less pronounced (Fig. 1c). The increase in contractility was followed by a decrease in contractility at high drug concentrations, resulting in similar or reduced contractility levels compared to untreated control tissues (Fig. 1a–c).

All other drugs also induced a concentration-dependent increase in *MAF*, *F* and *A* compared to untreated control tissues, but drug concentrations provoking significant effects differed between drugs. Statistically compared to lidocaine, bupivacaine induced a stronger increase in contractility which was followed by a more pronounced decrease in contractility at high drug concentrations (Fig. 2a–c). Contractility-enhancing effects of tetracaine did not differ significantly from lidocaine effects, but the decrease in contractility at high drug concentrations was stronger compared to lidocaine (Fig. 2a–c). Mexiletine effects on *F* (Fig. 2b) and *A* (Fig. 2c) showed only slight differences compared to lidocaine, but these effects summed up to significantly stronger effects on *MAF* (Fig. 2a). Procaine induced a more pronounced increase in contractility at medium–high to high drug concentrations, but a