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Lidocaine reduces tissue oedema formation in equine gut wall challenged by ischaemia and reperfusion

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Summary

Reduction of small intestinal propulsive motility is still a major problem after equine colic surgery. It is a common side effect after an ischaemia and reperfusion injury, frequently resulting in development of a postoperative ileus. Lidocaine is commonly used for prokinetic treatment in the early postoperative period. Determining the contractile performance of intestinal smooth muscle in vitro, it was shown that lidocaine increased contractility after an in vivo artificially induced short-term ischaemia and reperfusion injury. A lidocaine-dependent decrease in membrane permeability of smooth muscle cells indicated a possible mechanism for cellular repair effects. Aim of this study was to examine if structural alterations of intestinal gut wall induced by short-term ischaemia and reperfusion can be prevented by lidocaine treatment during reperfusion. Tissue slices from control and ischaemic and reperfused equine small intestine - untreated and treated with lidocaine – were prepared, stained and analysed. A classification protocol according to Snyder et al. (1998) was used to evaluate morphological alterations like filling of blood vessels, looseness of tissue, haemorrhage in the submucosa, and villus degeneration. Short-term ischaemia and reperfusion was able to induce increased blood vessel filling, enhanced looseness of mucosal and muscular layer, mild haemorrhage and slight degeneration of intestinal villi. Lidocaine significantly reduced the looseness of tissue in the submucosal and muscular layer, indicating prevention of interstitial oedema. This might be due to the membrane stabilising effect of lidocaine. Besides proven prokinetic features lidocaine could prevent structural alterations of gut wall induced by ischaemia and reperfusion.

Keywords: lidocaine, histomorphology, jejunum, ischaemia, reperfusion

Lidocain reduziert die Bildung eines Gewebeödemes in der ischämisch- reperfusionsgeschädigten Darmwand des Pferdes

Eine reduzierte propulsive Motilität des Dünndarmes tritt oft als Komplikation nach Kolikoperationen auf. Schäden durch Ischämie und Reperfusion sind mitverantwortlich für die Entwicklung eines postoperativen Ileus. Das Prokinetikum der Wahl in der postoperativen Phase ist Lidocain. Durch isometrische Kraftmessungen von in vivo durch Ischämie und Reperfusion geschädigtem Jejunum, das mit Lidocain behandelt wurde oder unbehandelt blieb, konnte in vitro gezeigt werden, dass Lidocain eine kontraktilitätssteigernde Wirkung auf die geschädigte glatte Muskulatur hat. Daneben wird die Freisetzung von Creatinkinase, einem Marker für die Zellmembranpermeabilität, signifikant herabsetzt. Ziel dieser Studie war es zu erfassen, ob strukturelle Veränderungen der Darmwand, die durch eine kurzzeitige Ischämie und Reperfusion ausgelöst werden, durch eine Behandlung mit Lidocain in der Reperfusionsphase verhindert oder abgeschwächt werden können. Hierzu wurden von ungeschädigten (Control), von durch Ischämie und Reperfusion geschädigten Darm (IR) und von durch Ischämie und Reperfusion geschädigtem und anschließend mit Lidocain behandeltem Darm (IRL) Proben entnommen. Von den Darmwandproben wurden histologische Schnitte angefertigt. Mit einem Bewertungssystem angelehnt an Snyder et al. (1998) wurde der Einfluss von Schädigung und Lidocainbehandlung auf morphologische Parameter wie Füllung der Blutgefässe, die Auflockerung des Gewebes, das Vorhandensein von Hämorrhagien sowie die Integrität der intestinalen Villi erfasst. Die Ergebnisse zeigen, dass schon eine kurzzeitige Ischämie und Reperfusion ausreichend sind, um eine erhöhte Blutfüllung der Gefäße, eine Auflockerung des Bindegewebes von Mukosa und Muskularis sowie milde Hämorrhagien und Degeneration der intestinalen Villi auszulösen. Lidocain verringerte die Auflockerung der intestinalen Schichten, was für eine reduzierte Ödembildung in behandeltem Gewebe spricht. Dies könnte auf der membranstabilisierenden Wirkung des Lidocains beruhen. Neben der bestätigten prokinetischen Wirkung reduziert Lidocain strukturelle Schäden der Darmwand, die durch Ischämie und Reperfusion ausgelöst werden.

Schlüsselwörter: Lidocain, Histomorphologie, Jejunum, Ischämie, Reperfusion

Introduction

Ischaemia and reperfusion, associated with intestinal strangulation and surgical reposition, can cause smooth muscle injury. Consequently, muscle cell function and contractile performance is decreased. As previously described, lidocaine improved basic cell functions and thereby muscle cell contractility, especially in ischaemia and reperfusion challenged smooth muscle (*Guschlbauer* et al. 2010). In general, restoring contractility of smooth muscle after ischaemia and reperfusion injury is essential for recovery of propulsive intestinal motility. Ischaemia, reperfusion, intraluminal distention and decompression decreases intestinal motility but also results in severe structural changes in all layers of the equine jejunum (*Dabareiner* et al. 2001, *Snyder* et al. 1988, *White* et al. 1980, *Weixiong* et al. 2005). *Freeman* et al. (1988) reported findings that brief periods of strangulation obstructions can induce severe changes in the jejunal wall. After two or three hours of an artificial induced ischaemia in the distal portion of the jejunum, necrosis was seen in the villus tip cells of the strangulated segment. This disintegration of texture resulted in exposing of lamina propria and capillaries. Further effects of ischaemia were oedema and haemorrhage in the interstitium. Structural changes after short-term strangulation in the gut wall and reduced contractility after ischaemia and reperfusion injury of the smooth muscle indicate the importance of morphological integrity of gut wall for intestinal motility (*Freeman* et al. 1988).

The observed decrease in smooth muscle function after 15 min of ischaemia and 15 min of reperfusion (*Guschlbauer* et

al. 2010) is most likely accompanied by morphological changes in the gut wall. The aim of this study was to evaluate, if 15 minutes of an in vivo artificially induced short-term ischaemia followed by 15 minutes of reperfusion were able to induce morphological changes in the jejunal wall. It was hypothesised that lidocaine is able to prevent these structural alterations thereby ameliorating the function of intestinal smooth muscle.

Material and Methods

Surgical procedure of ischaemia-reperfusion injury

Twelve adult warmblood horses of various breeds were used in this study. The 6 mares and 6 geldings aged from 3 to 22 years (450-555 kg bwt), were clinically healthy and showed no gastrointestinal disorders. Two weeks before surgery horses received an anthelmintic treatment and were kept in individual stalls with free access to hay and water. A modified jejunal ischaemia and reperfusion injury model was used as described previously (*Guschlbauer* et al. 2010). Before surgery the horses had to fast for 6 h. For premedication horses received 0.8-1.1 mg/kg bwt xylazine (i.v.). Anaesthesia was induced by 0.05 mg/kg bwt diazepam and 2.2 mg/kg bwt ketamine (i.v.). Balanced anaesthesia was maintained with isoflurane in 100% oxygen and continuous rate infusion of ketamine at 1 mg/kg bwt/h. Dobutamine, lactated Ringer's solution and hydroxyethyl starch were administered to maintain a mean arterial blood pressure above 60 mmHg during anaesthesia. After the induction of anaesthesia and tracheal intubation, the horses were positioned in dorsal recumbency. After aseptic preparation a routine laparatomy was performed. At the beginning a 25 cm segment of jejunum was resected and used as control tissue (Control). To initiate an ischaemia and reperfusion injury, mesenteric vessels of a 25 cm segment (IR), located in the distal jejunum about 1.5 m orally to the ileocaecal fold, were ligated using Penrose drains to maintain the integrity of the vessels. Gut lumen was also closed with Penrose drains. The intestinal segment was distended by infusion of body-warm Ringer's solution to 21 mm Hg intraluminal pressure (Fig. 1).

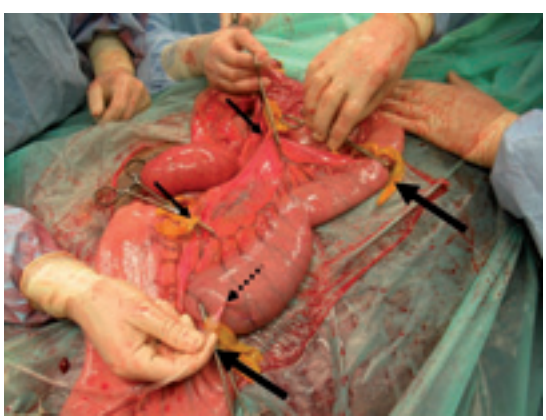

Abb. 1 Artifizielle Ischämie: Ein Segment des terminalen Jejunums wurde mit Hilfe von Penrose-Drains an beiden Seiten verschlossen (große Pfeile). Die zu- und abführenden Mesenterialgefäße sind zur Erzeugung einer transienten Ischämie ebenfalls mit Penrose-Drains verschlossen (kleine Pfeile). Die Instillation von körperwarmer Kochsalzlösung (gestrichelter Pfeil) diente der Distension des Darmes.
Artificial ischaemia: A segment of the distal portion of jejunum was closed on both sides using Penrose Drains (large arrows). Afferent and efferent mesenterial vessels were ligated using Penrose Drains to induce the transient ischaemia (small arrows). Body warm Ringer solution was instilled to distend the gut wall (dotted arrow).

Ischaemia was maintained for 15 min. Meanwhile jejunum was replaced into the abdominal cavity. Afterwards, ligations were removed and the intraluminal fluid was manually emptied once into the caecum. For reperfusion, the segment was replaced into the abdominal cavity again for another 15 min. Thereafter the segment was resected and both tissues were prepared for histological examination.

In vivo infusion of lidocaine

A further jejunal segment (IRL) was challenged exactly as it was described above for the ischaemia and reperfusion segment (IR). Immediately after ligation of mesenteric vessels and lumen closure, a loading bolus infusion lasting 10 minutes (1.3 mg/kg bwt i.v. lidocaine) was initiated. This was followed by a constant rate infusion of 0.05 mg/kg bwt/min lidocaine. For reperfusion, the segment was replaced into the abdominal cavity again for 15 min. Thereafter the segment (IRL) was resected and prepared for histological examination. Lidocaine was used as a 2% solution (bela pharm, Germany).

All horses were euthanized without regaining consciousness after surgery. Procedures were approved by the State Office for Consumer Protection and Food Safety in accordance with the German Animal Welfare Law.

Analysis of histomorphological alterations in IR injured intestinal wall

After exenteration of intestine (Control, IR and IRL), 1x1 cm pieces of total gut wall were taken, rinsed with Krebs-Henseleit-solution (pH 7.4) and pinned flat in a dissecting dish. Subsequently, they were fixed in 4% paraformaldehyde for 24 h. Thereafter fixed tissue samples were frozen in liquid propane to avoid tissue disintegration by bubbling as it occurs by freezing samples in liquid nitrogen directly. Thereafter, samples were further cooled down in liquid nitrogen and were stored at -80 °C until processing. Using a cryostat, tissue specimens were cut into 8 μm slices. Consecutive slices were mounted on glass slides. Tissue slices were stained with haematoxylin and eosin (HE) in a routine manner. For creating of images a light microscopy (4 x and 20 x magnification, IX70 microscope, Olympus Optical Co., LTD., Tokyo, Japan) was used. Photomicrographs of each slice for documentation and anlysis were taken (1 Pixel = 1.08 Microns; 4 x magnification) and analysed by software Leica® Application Suite 2.8.1 (Deutschland). Out of three different slices of each tissue per animal three randomly defined areas from each tissue slice were examined (n = 9 areas per tissue specimen (Control, IR, IRL) and horse).

The morphological integrity of gut wall was classified by using a protocol according to *Snyder* et al. (1998). Originally, using the Snyder protocol, the mucosa, submucosa and muscularis of sections were examined and graded regarding the extent of mucosal degeneration, presence of leucocytes, oedema, small focal separation of mucosal epithelial cells, and of haemorrhage. In this study, extent of blood vessel filling, oedema (looseness of tissue), haemorrhage, and of villus degeneration was assessed by using a similar 0 to 3 scoring system according to *Snyder* et al. (1998). The extent was classified according to the severity of alterations (0 negative: without findings; 1 mild: slightly observable, but not extensi-

ve; 2 moderate: extensive, but not converting normal relationships; 3 severe: extensive to harm physiological integrity).

Statistical analyses

Data were given as mean ± SEM of n = 12 horses. Each mean was composed of 9 values per horse for Control, IR and IRL injured smooth muscle. The significance of differences was calculated by using the One Way Analysis of Variance for matched observations and Tukey s multiple comparison test to detect significant differences between the groups. The level of significance was set at $p<0.05$. All statistical analyses were performed using software graph.pad.prism 4.0.

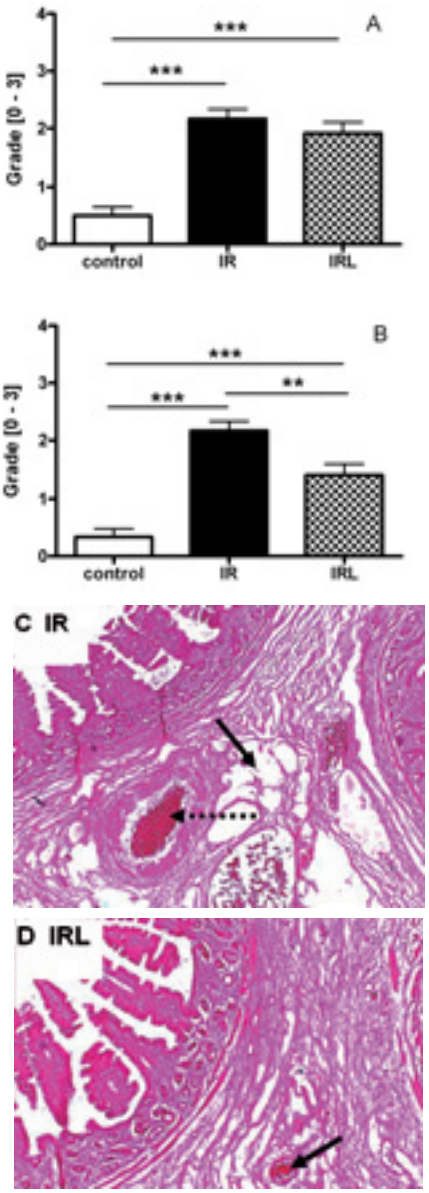

Abb. 2 A-D **A**. Grad des Füllungszustandes der Blutgefäße in der Submukosa in Gewebeschnitten von ungeschädigten (Control), geschädigten (IR) und mit Lidocain behandeltem Darm (IRL). **B**. Graduelle Auflockerung der Strukturen der Submukosa und der Muskularis in Gewebeschnitten von ungeschädigten (Control), geschädigten (IR) und mit Lidocain behandeltem Proben (IRL). Dargestellt in A. und B. sind Mittelwerte ± SEM, n=12, One Way ANOVA (repeated measures) $p<0.0001$, Tukey post test mit ***$p<0.001$, **$p<0.01$. **C**. Repräsentativer Gewebeschnitt des distalen Jejunums nach einer Ischämie und Reperfusionsschädigung (IR). Die Auflockerung der Tela submukosa (schwarzer großer Pfeil) ist charakteristisch für Ödembildungen. Die Blutgefässe sind dilatiert und mit Blutkörperchen gefüllt (großer gestrichelter Pfeil) **D**. Histologischer Schnitt des equinen distalen Jejunums nach einer Ischämie und Reperfusionsschädigung, das vor Beginn der Reperfusion mit Lidocain behandelt wurde (IRL). Die Auflockerung des Gewebes in der Tela submucosa ist gegenüber der Gruppe IR reduziert. Die Blutgefässe sind geringgradig dilatiert und mit Blutkörperchen gefüllt (großer Pfeil). Dargestellt in C. und D. sind Hämatoxylin/Eosin-Färbungen von IR- und IRL- Schnitten in 4facher Vergrößerung.
A. *Extent of filling of blood vessels in submucosa of undamaged gut (Control), injured gut (IR), and injured gut that was treated with lidocaine (IRL).* ***B****. Gradual looseness of tissue in the submucosa and muscularis of undamaged gut (Control), injured gut (IR) and injured gut that was treated with lidocaine (IRL). Mean ± SEM for n=12 horses are shown in Fig. A. and B., One Way ANOVA (repeated measures) $p<0.0001$, Tukey post test with ***$p<0.001$, **$p<0.01$.* ***C****. Representative slice of distal jejunum after an ischaemia and reperfusion (IR) injury. Looseness of submucosa (large black arrow) is indicating oedema. Blood vessels are enlarged and filled with blood cells (large dotted arrow).* ***D****. Representative slice of distal jejunum after an ischaemia and reperfusion injury that was treated with lidocaine before reperfusion (IRL). Looseness of submucosa and muscularis is in comparision to IR reduced by lidocaine treatment. Blood vessels are slighty enlarged and filled with blood cells (large arrow). C. and D. show Haematoxylin/Eosin stainings of IR and IRL slices in 4fold magnification.*

Results

After 15 minutes of ischaemia followed by 15 minutes of reperfusion with or without lidocaine treatment alterations in gut wall morphology were observed as analysed and classified according to *Snyder* et al. (1998).

Filling of blood vessels and haemorrhage in submucosa

Blood vessels of IR and IRL tissues showed a significantly increased amount of red blood cells in the lumen in comparison with the control group. Lidocaine treatment during ischaemia and reperfusion did not affect blood filling of vessels (Figure 2 A). Mild haemorrhage in submucosa as well as in the tunica mucosa was sometimes observable, but not extensive and without significant difference between control, IR and IRL tissues (data not shown).

Looseness of tissue

In IR tissues the extent of looseness of tissue in the submucosa and muscularis was significantly increased in comparison with the control tissues indicating slight to severe swellings. Lidocaine treatment was able to decrease extent of looseness significantly. A reconstruction of tissue structure to that of control tissue could not be achieved (Figure 2 B). HE stainings represented the looseness of the submucosa with enlarged empty spaces around the crypts and with broadening of mucosa, submucosa and muscularis (Figures 2 C (IR) and D (IRL)).

Villus degeneration

Villus degeneration was found in all three specimens, Control, IR and IRL. Even though this is the most difficult parameter to assess in histological specimens due to technical reasons, there was a slight significant increase in villus deterioration in IR samples ($p<0.05$). Lidocain treatment (IRL) see-

med to protect villi to degenerate, but this was not statistically significant (data not shown).

Discussion

The equine small intestine is responsible for the propulsive, directed movement of chymus by coordinated smooth muscle contractions whereby mucosa is absorbing nutrients from the lumen towards the mucosal vascular space simultaneously (*Grace* 1971). For both, motility and absorption morphological and functional integrity of intestinal wall is of vital necessity. Short-term ischaemia and reperfusion injury is able to decrease contractility in vitro significantly (*Guschlbauer* et al. 2010).

Additionally, the results of this histological study clearly showed that only 15 minutes of an artificial, in vivo induced ischaemia followed by 15 minutes of reperfusion were able to impair all layers of equine small intestine. The looseness of mucosa, submucosa, and muscularis indicated the formation of oedema in intestinal wall. Oedema formation was also observed in a study of *Whitehead* (1976). He reported that the earliest ultrastructural and histological changes observed in rat intestine, which was ischaemic for 5 to 10 minutes, included mitochondrial swelling and dilation of superficial mucosal capillaries with extravasations of fluid leading to oedema. Furthermore, he stated that intestinal distention and tissue pressure exceeded venous pressure quickly and increased capillary hydrostatic pressure thereby enhancing capillary filtration. As a consequence fluid accumulated in the lamina propria, resulting in oedema of intestinal wall as assessed by histological studies (*Allen* et al. 1988). The fluid accumulation in the submucosa and muscularis in the intestinal wall of horses may also be explained by enhanced capillary filtration due to the larger extent of blood vessel filling and higher capillary hydrostatic pressure. Additionally, the increase in filtration rate could be supported by an increase in membrane permeability of capillary endothelial cells due to ischaemia-reperfusion injury. Higher membrane permeability was found in intestinal smooth muscles cells after short-term ischaemia and reperfusion injury (*Guschlbauer* et al. 2010). Reperfused jejunal segments expressed a hyperaemic response with mild to moderate filling of blood vessels with erythrocytes and neutrophils in this study. This may have been caused by the strong blood reflow after the ischaemic period. The same observation was made by Dabareiner et al. (1993) after a period of 70 minutes of ischaemia followed by 60 minutes of reperfusion. However, significant submucosal haemorrhage with blood cell release into the extravasal compartment was not provoked by the strong capillary filling.

Lidocaine was able to decrease the formation of oedema significantly without any influence on blood vessel filling in IR injured intestinal tissues. High capillary pressure was most likely maintained in the gut wall treated with lidocaine in vivo. However, as known from a former study, lidocaine was able to decrease membrane permeability of smooth muscle cells as assessed by the diminished release of Creatin kinase (*Guschlbauer* et al. 2010). If lidocaine was able to decrease membrane permeability in general, the stabilization of endothelial cell membranes should decrease leakage of vessels and may thereby prevent oedema formation in small intestinal gut wall. Therefore, lidocaine was able to decrease structural alterations in ischaemic and reperfused jejunal intestine, though reconstruction of structural conditions of the intestinal tissues to the control level could not be observed.

One possible cellular mechanism of lidocaine effects could be the inhibition of superoxide radicals in ischaemia and reperfusion injured tissues (*Rimbäck* et al. 1990). Superoxide radicals were discussed to be the reason for progressive damage of equine intestinal tissue during reperfusion (*Snyder* et al. 1988, *Lunegren* and *Haglund* 1978). Immediate treatment with lidocaine during intestinal wall reperfusion may inhibit the formation of superoxide radicals and therefore prevent mucosal damage.

To conclude, the exact pathways by which lidocaine prevents or repairs gut wall structural and functional alterations are unknown so far. However, lidocaine is most likely able to prevent and repair structural alterations derived from ischaemia and reperfusion injury.

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