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The relationship between morphology and disaccharidase activity in ischemia — reperfusion injured intestine

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Background: Questions regarding functional markers characterizing injured intestines remain unanswered. Brush border disaccharidases are crucial for the functioning of the intestines. Aims: The study was designed to assess changes in disaccharidase activity (DA) following intestinal injury and to compare them with morphological changes. Methods: Wistar rats, randomly divided into six experimental groups (each n = 6), were subjected to different ischemic/reperfusion injury. One-hour mesenteric ischemia followed by reperfusion for 0, 1, 2, 4, 12 or 24 hours was induced. As a control group sham-operated animals were used (n = 6). Intestine morphology was evaluated using histopathological injury index (HII) and goblet cell (GC) detection. DA (sucrase and maltase) was studied in mucosal scrape or in entire intestinal wall samples. Results: Moderate morphological damage (HII, GC) after mesenteric ischemia was detected. Deepening of the injury was found during reperfusion with a maximum after two hours. Improved morphology with longer reperfusion confirmed reversible damage with almost normal mucosal structure after 24 hours of reperfusion. Similar pattern was observed when DA was measured. The lowest activity was detected after 2 hours of reperfusion followed by increasing activity in the subsequent reperfusion periods. Physiological values after 24 hours of reperfusion were seen only in samples of entire intestinal wall. Conclusions: Significant changes in intestinal DA were observed after intestinal ischemia/reperfusion injury. A similar pattern was seen for morphological characteristics. Although based on microscopic survey the intestine seems to be fairly regenerated, some functional limitation is expected to persist.

Key words: disaccharidase, small intestine, ischemic/reperfusion injury, brush border, morphology

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INTRODUCTION

Sufficient functional status of transplanted intestines is necessary for successful intestinal transplantation (IT). Several studies confirming extreme small bowel vulnerability have been published recently.

The low tolerance of insult of different kinds and range (i.e. mechanical manipulation, ischemia and reperfusion) causes relatively short "viability" of this organ. Mesenteric ischemia leads to marked jejunal damage that subsequently deepens during reperfusion — the oxygen paradox (Su, 1998). Strong interest in the goblet cell (GC) population in the last few years has produced significant results. Their endogenous activity allows them to support epithelial restitution and facilitate migration of cells, and also through specific intestinal trefoil factor peptides they are able to block apoptosis (Taupin & Podolsky, 2003; Kjellev, 2009). Our previous studies (Tóth *et al*., 2007; Varga *et al*., 2009, 2011) described in detail the morphological changes at the level of particular cell populations in the intestines during IT (graft harvest, preservation, implementation and reperfusion) have been described in detail (Varga *et al*., 2009). Knowledge of concurrently arising functional changes could bring useful information.

Studies concerning disaccharidase activity (DA) in the intestine have produced discrepant results. While some experiments found a correlation between DA and morphology, other studies have refuted that fact (Gupta *et al*., 1999; Arcuni *et al*., 2000; Vieira *et al*., 2000; Huang *et al*., 2005; Li *et al*., 2006; Oltean *et al*., 2007; Tori *et al*., 2007; Prasad *et al*., 2008; Cuong *et al*., 2011). Despite these discrepancies, it is accepted that DA decrease resulting from pathological situations leads to poor nutrition status (Gebhard *et al*., 1983; Tran *et al*., 2011). Fifteen years ago Plushke *et al*. referred to DA as a suitable marker for detection of enterocyte maturity and functional capacity (Pluske *et al*., 1996). It was confirmed by other studies that weaning is associated with marked changes in the histology and biochemistry of the intestines, and such changes include increased cell proliferation and differentiation (Lin *et al*., 1998), and altered activity of the brush border disaccharidases, sucrase and lactase (Šabat & Veloso, 2003).

The discrepancies in the previous results demonstrate the necessity of exact knowledge regarding DA changes induced by pathological insults in the small intestine. Correlation accuracy between DA and morphological changes remains unaddressed (Gupta *et al*., 1999). The aim of the present study was to assess DA at different extents of intestinal damage, and to corelate it with the morphological changes in different cell populations as a potential functional marker of intestinal damage.

METHODS

This experiment was approved by the Committee for Ethics on Animal Experiments at the Faculty of Medi-

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Abbreviations: DA, disaccharidase activity; HII, histopathological injury index; GC, goblet cell.

cine, P.J. Šafárik University, Košice, Slovakia and the experimental protocol was approved by the State Veterinary and Food Administration of Slovak Republic No. 72 2843/08-221a.

Design of the experiment. In this experiment, 42 healthy male Wistar rats weighing 250 ± 30 g were used. All the animals were housed in standard conditions with free access to water and commercial chow and laboratory temperature of 21°C. After 5 days of quarantine the animals were fasted overnight in cages with raised floors to minimize coprophagy. Water was provided ad libitum. Random distribution into 6 experimental groups (J0, J1, J2, J4, J12 and J24; each n = 6) was carried out, and in the sham-operated control (SO; n = 6) biopsies of intact jejunum were taken immediately after midline laparotomy.

The animals were anesthetized with intraperitoneal injection of ketamine 60–80 mg/kg (Narketan 10 inj. ad us. vet., Vétoquinol S.A., Lure Cedex, France), and xylazine 8–10 mg/kg (Xylariem inj. ad us. vet., Riemser Arzneimittel, Greifswald-Insel Riems, Germany). After 1 hour 1/3 of the total dose was added intramuscularly. During the whole procedure body temperature was maintained by a heating pad set to 37°C. Using sterile techniques, 6 cm midline laparotomy was carried out. The small intestine was carefully mobilized to minimize injury induced by mechanical manipulation, subsequently the cranial mesenteric artery (CMA) was isolated and ischemia was induced by its occlusion using an atraumatic microvascular bulldog-clamp for 1 hour. At the end of ischemia the artery was unclamped, and this was followed by periods of reperfusion in accordance with the experimental design. The sham-operated animals underwent laparotomy with isolation of CMA without its clamping and immediately the intact samples were taken. The abdominal incision was closed in two layers with Silon 2.0 EP suture (Chirmax, Prague – Modřany, Czech Republic) for all operations. After the given reperfusion period, the animals were sacrificed and jejunal samples were taken at 0, 1, 2, 4, 12 and 24 hours of reperfusion for the experimental groups J0, J1, J2, J4, J12 and J24, respectively.

Morphological assessment. Bioptic samples of the jejunum, 1–2 cm long, were taken after the prescribed reperfusion periods 10 cm from the ligament of Treitz. Harvested samples were immediately rinsed in cold saline and fixed in 4% para-formaldehyde. The tissues were then embedded in paraffin, cut into 4-5 μm sections, and mounted. The histopathological injury index (HII) of the intestine was examined blindly, and ischemia-reperfusion (IR) injury was evaluated on a grading scale from 0 to 8 according to the Park/Chiu scoring system adapted by Quaedackers *et al.* (2000), as we have previously described (Varga *et al.*, 2010). This index is based on the extent and severity of histopathological abnormality or lesion in the section; briefly: 0 = normal mucosa; 1 = subepithelial space at villus tip, often with capillary congestion; 2 = more extended subepithelial space with moderate epithelial lifting; 3 = epithelial lifting along villus sides; 4 = denuded villi; 5 = loss of villous tissue; 6 = crypt layer destruction; 7 = transmucosal infarction; 8 = transmural infarction.

Histochemical analysis of goblet cell population. The population of goblet cells (GC) present in the intestinal mucosa was detected using the alcian blue histochemical staining method. Alcian blue 8GX solution (pH 2.5; Sigma-Aldrich, St. Louis, MO, USA) stains both sulphated and carboxylated acid mucopolysaccharides and sulphated and carboxylated sialomucins (glycoproteins). Excessive amounts of non-sulphated acidic mucosubstances were seen in the cytoplasm of secretory GC. Strongly acidic mucosubstances in the cytoplasm were stained blue, while nuclei were counterstained pink to red by nuclear red stain. Alcian blue/neutral red-stained tissues were acquired, and the number of alcian blue- positive GC was determined in 10 intestinal villi in each sample.

All the tissue sections were examined and photographed using an Olympus BX50 light microscope with Olympus SP350 camera (Olympus, Tokyo, Japan) and were evaluated blindly by two independent histologists.

Disaccharidase activity assay. Two main disaccharidases, maltase and sucrase, were chosen to assess the functional status of the injured intestine. The bioptic samples for biochemistry were obtained either as scrape of the mucosa or as entire intestinal wall from the proximal part of the jejunum where the biopsies for morphology assessment were taken. The samples of mucosal scrape were taken using a scalpel. Careful scraping of the epithelium including crypts up to tela submucosa was carried out. The samples of entire intestinal wall were then harvested from an adjacent intestinal segment. The samples of small intestine (2 cm long) were homogenized in saline solution (500 μl) by mechanical homogenizer T10 basic ULTRA-TURRAX (IKA, Germany). During homogenization the samples were chilled in ice. Homogenate (60 μl) was diluted 1:20 with saline (1020 μl). Diluted samples of homogenate (50 μl) were pipetted into Eppendorf tubes for measurement of sucrase and maltase activity with addition of an appropriate disaccharide substrate (50 μl). The mixture was incubated at 37°C for 60 minutes in a thermoblock. The enzymatic reaction was stopped at 100°C. The content of released glucose was determined using the enzymatic glucose oxidase Bio-La-Test (Lachema, Brno) by adding 1 ml of working solution (glucose oxidase, peroxidase, 3-methylphenol, 4-aminophenazone, phosphate buffer pH = 8). The mixture was incubated at 37°C for 15 minutes and absorbance was read at λ = 490 nm against a blank containing 1 ml of working solution for measurement of glucose and distilled water (100 μl). The DA, i.e.; the activities of sucrase and maltase, were assessed following Dahlqvist (1968). Protein content in homogenates (10 μl) was determined with Briliant Blue dye (1 ml) at λ = 595 nm according to Bradford (1976). The specific activity was calculated as the activity divided by the concentration of total protein. The activities of disaccharidases were expressed as μkat/mg of protein.

Statistical analysis. The statistical analysis was performed using GraphPad InStat version 3.01 (GraphPad Software, San Diego, CA). Semi-quantitative results (histopathological injury index, HII) were set as medians with 50th percentile intervals. Time-dependent changes were analyzed using Friedman's non-parametric test with post-test for related samples, and box plots show median (50th percentile), inter-quartile range (lower and upper quartiles), maximum and minimum values. Quantitative results (goblet cell, disaccharidase activity) were evaluated using one-way ANOVA with a multiple-comparison Tukey-Kramer post hoc test. All the results are expressed as mean ± SEM. P values less than 0.05 were considered significant.

RESULTS

Morphological characteristics

Nearly intact jejunal mucosa according to HII was detected in the sham-operated control group SO (SO = 0.21 ± 0.08). One hour of oxygen deprivation alone

Table 1. Intestinal morphology and disaccharidase activity.

Group	Histological analysis		Disaccharidases analysis			
	HII	GC number	Maltase activity (µkat/mg protein)		Sucrase activity (µkat/mg protein)	
			Mucosa	Entire wall	Mucosa	Entire wall
SO	0.21 ± 0.08	40.3 ± 2.07	55.19 ± 2.32	1.3 ± 0.27	5.11 ± 0.32	1.75 ± 0.24
J0	2.38 ± 0.63	29.19 ± 2.02	19.8 ± 0.99***	0.83 ± 0.07	2.27 ± 0.42**	1.39 ± 0.16
J1	4.2 ± 0.1***	13.7 ± 2.92**	12.67 ± 0.01***	0.77 ± 0.16	2.02 ± 0.01**	1.33 ± 0.04
J2	4.5 ± 0.21***	11 ± 1.98**	11.43 ± 0.67***	0.66 ± 0.08	1.82 ± 0.64***	1.09 ± 0.06*
J4	4.13 ± 0.63	20.33 ± 2.91**	14.4 ± 1.45***	0.99 ± 0.19	1.62 ± 0.31***	1.49 ± 0.19
J12	1 ± 0.1	30.47 ± 0.77	16.38 ± 1.86***	1.1 ± 0.22	2.37 ± 0.25**	1.5 ± 0.17
J24	0.44 ± 0.06	31.2 ± 2.02	18.37 ± 3.92***	1.12 ± 0.02	2.39 ± 0.74**	1.76 ± 0.06

*$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$

caused moderate morphological damage (J0 = 2.38 ± 0.63) expressed by local epithelial lifting along villus sides. Significant mucosal damage was detected in the reperfusion periods, i.e., after one hour of reperfusion (J1 = 4.2 ± 0.1; $p < 0.001$ J1 *vs* SO), with a maximum damage after 2 hours after starting of reperfusion (J2 = 4.5 ± 0.21; $p < 0.001$ J2 *vs* SO). Denuded villi and loss of villous tissue were recorded, but the crypt compartment of the lamina propria mucosae was not affected. This could be considered as reversible damage, which was confirmed in the following reperfusion periods by a decrease of the HII to an almost normal mucosal structure after 24 hours of reperfusion (J24 = 0.44 ± 0.06; $p < 0.001$ J24 *vs* J1; $p < 0.001$ J24 *vs* J2) (Table 1, Fig. 1, Fig. 2).

One hour of anoxia led to a decrease in GC numbers in comparison with the sham- operated control group SO, where physiological numbers were detected (SO = 40.3 ± 2.07; J0 = 29.19 ± 2.02). A rapid depletion of GC was found immediately after reoxygenation (J1 = 13.7 ± 2.92; $p < 0.01$ J1 *vs* SO), and the lowest number of GC was observed after 2 hours of reperfusion (J2 = 11 ± 1.98; $p < 0.01$ J2 *vs* SO). Thereafter a progressive increase in GC numbers was recorded, reflecting intestinal mucosa regeneration. A GC population reduced by almost 25% compared to control was observed after

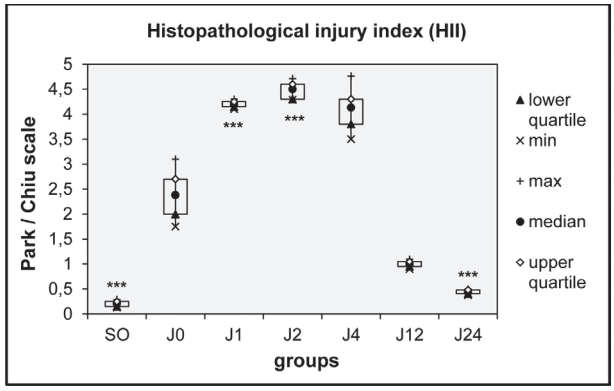

Figure 1. Histopathological injury index (HII) of intestines subjected to IR injury after H&E staining (*$p<0.001$ SO *vs* J1, SO *vs* J2, J24 *vs* J1 and J24 *vs* J2).**

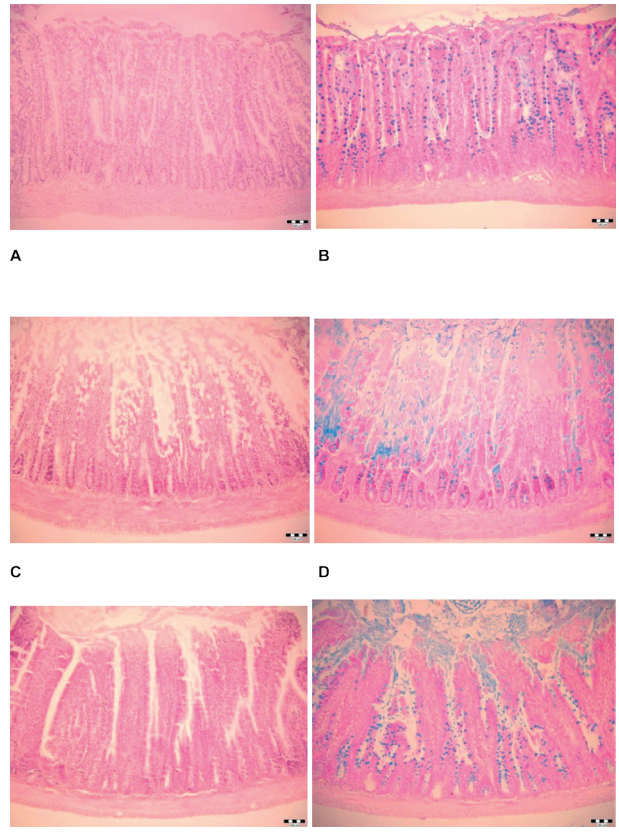

Figure 2. Microphotographs showing histopathological and histochemical findings
(A) Normal histology of intestinal wall in sham-operated control group (SO, H&E). **(B)** Normal distribution of goblet cell population in control group (SO, Alcian blue & Nuclear red). **(C)** Massive lifting down of intestinal villi as well as some denuded villi after 1 h of reperfusion (J1, H&E). **(D)** Disruption of goblet cell population after 1 h of reperfusion (J1, Alcian Blue & Nuclear red). **(E)** Regeneration of intestinal mucosa after 24 h of reperfusion (J24, H&E). **(F)** Renewal of mucin-producing goblet cells after 24 h of reperfusion (J24, Alcian blue & Nuclear red). Scale bars (A–F) represent 50 µm.

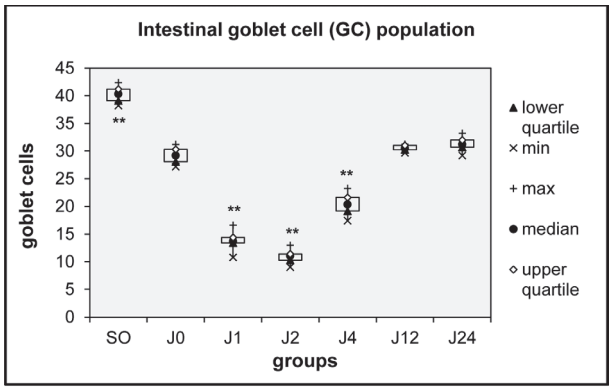

Figure 3. Goblet cell (GC) population in experimental and SO groups after Alcian Blue staining ($p<0.01$ SO *vs* J1, SO *vs* J2 and SO *vs* J4).**

24 hours of reoxygenation (J24 = 31.2 ± 2.02), but the difference with the SO group was not statistically significant. (Table 1, Fig. 2, Fig. 3).

A significant decrease in sucrase activity measured in mucosal scrape samples was detected immediately after one hour of mesenteric ischemia relative to the physiological value measured in the SO group (SO = 5.11 ± 0.32 μkat/mg; J0 = 2.27 ± 0.42 μkat/mg; $p<0.01$ J0 *vs* SO). A gradual decrease in the activity was observed during reperfusion, reaching a minimum at 4 hours of reperfusion (J4 = 1.62 ± 0.31 μkat/mg; $p<0.001$ J4 *vs* SO). Thereafter the activity increased slightly to approximately half of the control value (J24 = 2.39 ± 0.74 μkat/mg; $p<0.01$ J24 *vs* SO). The differences between the experimental groups at various time periods of reperfusion were not significant.

A similar course was observed for the maltase activity measured in the mucosal scrape. One-hour ischemia led to a significant decrease (J0 = 19.8 ± 0.99 μkat/mg) when compared to the SO group (SO = 55.19 ± 2.32 μkat/mg; $p<0.001$ J0 *vs* SO). The decrease in the enzyme activity continued through the first hours of reperfusion (J1 = 12.67 ± 0.01 μkat/mg; $p<0.001$ J1 *vs* SO), with the lowest value at 2 hours following oxygen supply restoration (J2 = 11.43 ± 0.67 μkat/mg; $p<0.001$ J2 *vs* SO). Then a gradual moderate increase was observed, although the values reached were still significantly lower than in the SO group. After 24 hours of reperfusion, the maltase activity was still significantly lower than in the SO group (J24 = 18.37 ± 3.92 μkat/mg; $p<0.001$ J24 *vs* SO). The differences between the experimental groups at various time periods were not significant.

Almost the same character of changes was observed when the DA was measured in samples of entire intestinal wall. Regarding sucrase, a decrease in comparison to the SO group (SO = 1.75 ± 0.24 μkat/mg) was detected immediately after declamping (J0 = 1.39 ± 0.16 μkat/mg). After reperfusion started the decline continued (J1 = 1.33 ± 0.04 1.09 ± 0.06 μkat/mg; $p<0.05$ J2 *vs* SO) followed by a slow increase in the remaining groups. A physiological activity of sucrase was regained after 24 hours of reperfusion (J24 = 1.76 ± 0.06 μkat/mg).

The activity of maltase in entire intestinal wall samples of the SO group was similar to that observed for sucrase (SO = 1.3 ± 0.27 μkat/mg). The course of changes durin the various periods of reperfusion was similar as well. A prominent decrease in the activity was detected at the beginning of reperfusion (J0 = 0.83 ± 0.07 μkat/mg). The progressing damage due to reperfusion was expressed by a further decrease in maltase activity during the first reperfusion periods, with the lowest value after 2 hours of reperfusion (J2 = 0.66 ± 0.08 μkat/mg), and then a gradual increase in maltase activity with increasing time of reperfusion. Near physiological values were observed at the end of reperfusion (J24 = 1.12 ± 0.02 μkat/mg). Despite that tendency, the differences between the experi-

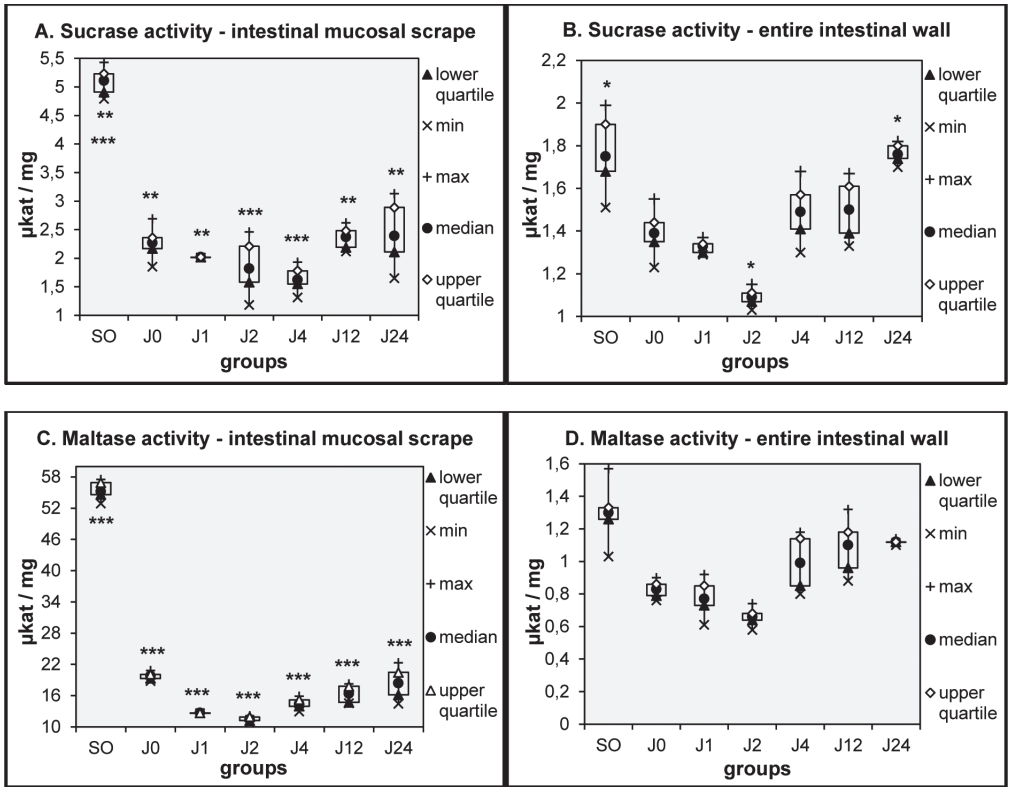

Figure 4. Activity of disaccharidases, sucrase and maltase, in samples of mucosal scrape and entire intestinal wall. **(A)** Sucrase activity in mucosal scrape (**$p<0.01$ SO *vs* J0, SO *vs* J1, SO *vs* J12 and SO *vs* J24; ***$p<0.001$ SO *vs* J2 and SO *vs* J4). **(B)** Sucrase activity in samples of entire intestinal wall (*$p<0.05$ SO *vs* J2 and J2 *vs* J24). **(C)** Maltase activity in mucosal scrape samples (***$p<0.001$ SO *vs* J0, SO *vs* J1, SO *vs* J2, SO *vs* J4, SO *vs* J12 and SO *vs* J24). **(D)** Maltase activity in samples of entire intestinal wall.

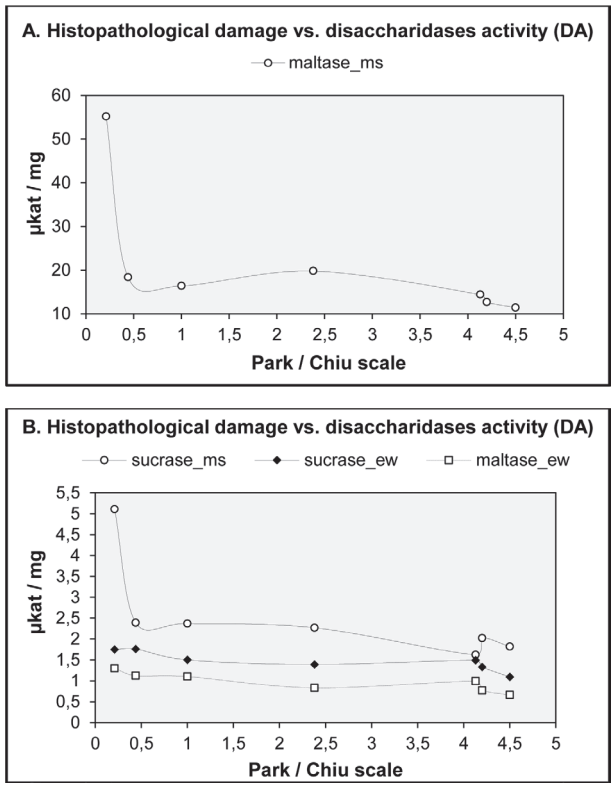

Figure 5. Relationship between morphological and functional characteristics of injured intestines.
(A) Histopathological injury index (HII) *vs* maltase activity in mucosal scrape samples (maltase_ms). **(B)** HII *vs* sucrase activity in samples of mucosal scrape (sucrase_ms) and samples of entire intestinal wall (sucrase_ew), and maltase activity in entire intestinal wall samples (maltase_ew).

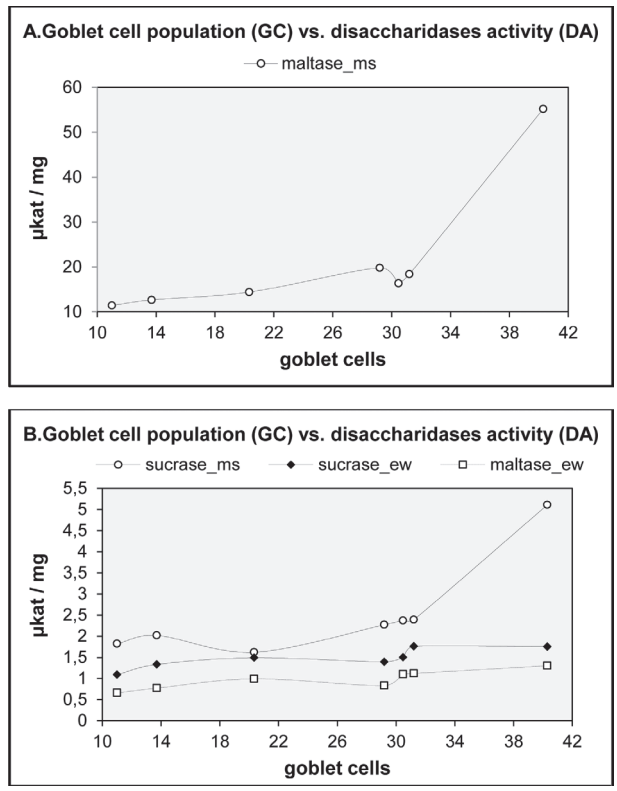

Figure 6. Relationship between morphological and functional characteristics of injured intestines.
(A). Goblet cell (GC population) *vs* maltase activity in mucosal scrape samples (maltase_ms). **(B)** GC *vs* sucrase activity in samples of mucosal scrape (sucrase_ms) and samples of entire intestinal wall (sucrase_ew), and maltase activity in entire intestinal wall samples (maltase_ew).

mental groups and the SO group were not statistically significant (Fig. 4).

Correlation between morphological and functional characteristics

Similar correlation was observed between HII, GC and DA. The injury after one hour of ischemia was expressed by an increased HII, a decrease in GC numbers as well as decrease in DA, both in the mucosal scrape and the entire wall samples. The deepening of the injury due to reperfusion in the next two hours was confirmed by further alteration in mucosal morphology and DA decrease. The improvement of tissue morphology and enhancement in DA in subsequent reperfusion periods showed the expeditious start of regeneration processes. Near physiological values of HII and GC were achieved after 24 hours of reoxygenation, while physiological intestinal wall (Fig. 5, Fig. 6).

DISCUSSION

This pilot study was conceived to observe the relationship between DA changes and the degree of morphological damage in the intestine suffering IR injury.

The changes in intestinal morphology resulting from IR injury of the small bowel have been documented clearly by many authors (Stallion *et al.*, 2002; Yildiz *et al.*, 2009 and 2010; Varga *et al.*, 2010; Grootjans *et al.*, 2010). In our experiment almost complete regeneration was observed after just 24 hours of reperfusion, suggesting an extraordinary reparative-regenerative capacity of the intestines when reversible damage is inflicted (Varga *et al.*, 2010).

The GC function of mucus production and thus the first-line protective barrier manifests the extreme importance of these defence cells. Kim and Ho (2010) have shown that not just secretory mucin glycoproteins as a major component of the mucus layer but also bioactive molecules such as epithelial membrane-bounds mucins play important roles in mucosal protection. As early as 1995, Taguchi *et al.* showed that a decrease in GC population and villus cell mucus content during intestinal graft preservation is an important sign of poor graft viability. Recently we have confiromed this fact in our study of GC changes during cold storage of intestines. With a lengthening time of preservation, graft mucosal alteration increased at a rate which correlated with the decrease in GC numbers (Varga *et al.*, 2011). In the present study, the tendency in GC development during intestinal IR injury was similar to HII. The reduction in GC is in close connection with disintegration due to epithelial lifting along the villous sides, denudation of the villi, and loss of villous tissue (Tóth *et al.*, 2011). Ikeda *et al.* (2002) clearly demonstrated that GC resist being detached along with the enterocytes from the villi subjected to short-term injury. Such higher resistance of GC could be partially explained by differences in the integrin family of cell surface receptors between GC and absorptive enterocytes (Perreault *et al.*, 1996). In any case, the relatively high resistance of GC to ischemic injury and the production of trefoil factor peptides with a direct influence on the epithelial restitution make GC a very important part of the intestinal healing processes.

The cleavage of oligosaccharides and disaccharides takes place in the small intestine through α-glycosidases. Brush border glycosidases are transmembrane glycoproteins passing through the whole membrane of enterocytes. The α-glycosidases (sucrase, maltase, lactase, and trehalase) are located in the brush border of the jejunum and part of the ileum, either separately or in complexes. The lowest activities are found at the crypts and increase continuously to the villus tips (Porter *et al*., 1982). Primarily, ischemia causes blockage of the ATP supply and initiation of catabolic processes. One of the first changes in this phase is the release of the tight junction and thus also disruption of the continuity of surface epithelial cell (Varga *et al*., 2011). The majority of the catabolic reactions are catalysed by enzymes activated through calcium cations, and so ischemia leads also to releasing of calcium cations. The calcium activates phospholipase, disturbing the integrity of the cytoplasmic membrane and thus also affecting disaccharidases. The third mechanism of the damage is production of oxygen and nitrogen radicals, mainly during reperfusion (Yeh *et al*., 2000). These pathological processes affect mucosal morphology as well as DA.

Two kinds of bioptic samples are usable for DA detection in the intestines, the mucosal scrape and the entire intestinal wall. The activity of disaccharidases is expressed as μkat/mg of total protein, thus when the entire intestinal wall is used, higher amounts of protein are expected in samples. On the other hand, technically easier sample harvesting is involved when a scrape of mucosa is taken with no (Aramayo, 1983; Raul, 1988).

A clear variance regarding different disaccharidase types can be observed. While in a population sucrase and maltase show consistent activity, the activity of lactase varies depending on various factors, for example age. These facts were confirmed by previously undertaken studies, where differences in lactase activity were verified while in the case of sucrase and maltase consistent values were detected (Teitelbaum *et al*., 1989; Fernandez *et al*., 1997; Vieira *et al*., 2000). For that reason lactase activity was not studied in this experiment.

In our experiment, DA was significantly affected by IR injury, in particular in mucosal scrape samples. Altered morphology after ischemia causes rapid decrease in monitored DA, which was even more prominent during the first hours of reperfusion. The peak of the damage based on HII and GC population was reached 2 hours after reoxygenation. Clear violation of the jejunal brush border as well as a substantial decrease in DA was seen. The lack of appropriate environment caused the lowest DA, both for sucrase and maltase in both types of samples. Prolonged reperfusion provided for improved morphology (HII and GC) as well as DA (maltase and sucrase). GC showed an evident increase in numbers due to their higher resistance to IR injury, although after 24 hours of reperfusion they still did not reach the control values (40.3 *vs* 31.2) thus showing incomplete mucosal restoration. After reaching the lowest values (J2 group) the HII increased during further reperfusion. The improved HII was expressed mostly by "crawling" of remaining enterocytes, not by their proliferation or maturation. This indicates that the morphology status expressed by HII is underscored as, "crawling" of enterocytes represents something like mimicry increase in brush border disaccharidases was seen, but in mucosal scrape samples they remained significantly lower when compared to the SO controls. At the end of the experiment, at 24 hours of reperfusion, the levels of both measured enzyme activities in samples of entire intestinal wall were nearly normal, reflecting the nearly restored intestinal morphology, while in the mucosal scrape samples they remained still significantly affected. These reductions could be caused by prolonged time of functional differentiation and maturation of enterocytes, as during the first 24 hours after ischemia the "crawling" of enterocytes was the main source of HII improvement. Thus still lower than physiological values of disaccharidases were detected in mucosal scrape samples, accurately showing the status of intestinal mucosa. By contrast, in samples of entire intestinal wall, near physiological values were measured. The inclusion of all crypts, where enterocyte proliferation starts, in samples of entire intestinal wall probably ensured the physiological values found. The higher protein concentration as well as affecting of crypts in entire intestinal wall samples caused lower DA comparing to mucosal scrape samples. Similar activities of maltase and sucrase have been measured also in a study of Oku *et al*. (2011) where samples of mucosal scrape were used (Oku *et al*., 2011). Therefore samples of entire intestinal wall are inappropriate for determination of DA. Since the villi (mainly at the top) remained still devoid of new enterocytes even after one day of reperfusion, significantly decreased values were detected in mucosal scrape samples. Longer reperfusion will lead to the continuation of enterocyte proliferation and maturation eventually producing physiological values of DA. An indirect evidence of correlation between HII and DA was provided by Oltean *et al*. (2007) using pre-treatment of were observed in comparison to non-treated grafts. Promising results of DA changes regarding intestinal graft rejection have been shown in other studies, although discrepant results are seen as well. While Kaufman *et al*. (1998) showed that assessment of mucosal disaccharidase activity provides no additional useful information concerning efficacy of anti-rejection therapy as compared to histologic analysis alone, other studies recommend DA assessment as an appropriate marker for study and verification of acute (Kuusanmäki *et al*., 1996) as well as chronic rejection (Kuusanmäki *et al*., 1997). Notably a correlation with morphological status was detected for sucrose and maltase, but not when lactase was measured (Teitelbaum *et al*., 1989). Simultaneous changes in DA and morphology of the small intestine can also be induced by other states where an IR insult is not present. Nowadays, the influence of dietary changes is the main focus of experimental studies regarding disaccharidases, producing discrepant data as well. A few such studies have found changes in morphology as well as in DA (Duff & Ettarh, 2002; Payne *et al*., 2006; Hedemann *et al*., 2006; Tran *et al*., 2011). On the other hand, there are papers showing no structural changes despite alterations in DA (Fernandez *et al*., 1997; Vieira *et al*., 2000). Studies of ethanol influence on DA give discrepant results as well. Whereas Huang *et al*. (2005) observed decrease in DA and increase in jejunal lipase with no histological changes with long-term ethanol consumption, other studies did not confirm that fact (Cunningham & Spach, 1987; Rodriguez-Castilla *et al*., 1996). An in vitro study has confirmed that ethanol at low concentrations (1–3%) may increase DA (Nano *et al*., 1990).

The obtained results show a close correlation between the morphological status and DA in IR injured intestine. Maturation and proliferation of enterocytes is probably the key point of DA renewal. The well-known behavior of GC has been confirmed in our study, correlating with the "crawling" of enterocytes. The determination of in-

testinal morphology using just HII leads to the mucosal underscoring. Thus the state of DA could provide information about intestinal mucosal renewal, mainly reflecting the enterocyte population. Mucosal scrape samples seem to be better suited for the assessment of intestinal disaccharidases, sucrase and maltase, than intestinal wall samples.

CONCLUSION

A limited numbers of studies report DA investigation. It has previously been clearly shown that pathological insults lead to changes in DA. Inconsistencies and discrepancies in reported data demand clarification regarding DA changes arising from pathological attacks. The knowledge of correlation between morphological changes and DA has crucial importance for subsequent experiments. The IR syndrome as a classical pathological situation leads to alteration in morphology as well as DA changes. For detailed investigation of the relationship between morphology and functional parameters, further studies are needed. It seems important to monitor DA activity, particularly maltase and sucrase, in mucosal scrape samples, which give more precise information about the functional condition of the injured intestine. Although based on microscopic survey the intestine seems to be fairly regenerated, functional limitations can be assumed to persist.

Disclosure

The authors report no conflicts of interest in this work.

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REFERENCES

Aramayo LA (1983) Disaccharidase activities in jejunal fluid. *Arch Dis Childhood* **58**: 686–691.

Arcuni J, Wang L, Franson R, Sonnino RE (2000) Modification of disaccharidase activity measurement in rat bowel. *J Invest Surg* **13**: 29–33.

Bradford M (1976) A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254.

Cunningham CC, Spach PI (1987) The effect of chronic ethanol consumption on the lipids in liver mitochondria. *Ann N Y Acad Sci USA* **492**: 181–192.

Cuong DT, Johanna C, Cory JX (2011) Dietary zinc and metallothionein on small intestinal disaccharideses activity in mice. *World J Gastroenterol* **17**: 354–360.

Dahlqvist A (1968) Assay of intestinal disaccharidases. *Anal Biochem* **22**: 99–107.

Duff M, Ettarh RR (2002) Crypt cell production rate in the small intestine of the zinc-supplemented mouse. *Cells Tissues Organs* **172**: 21–28.

Fernandez MIM, Galvao LC, Bertolozzi MF, Oliveira WP, Zucoloto S, Bianchi MLP (1997) Disaccharidase levels in normal epithelium of the small intestine of rats with iron deficiency anemia. *Braz J Med Biol Res* **30**: 849–854.

Gebhard RL, Karouani R, Prigge WF, McClain CJ (1983) The effect of severe zinc deficiency on activity of intestinal disaccharidases and 3-hydroxy-3-methylglutaryl coenzyme A reductase in the rat. *J Nutr* **113**: 855–859.

Grootjans J, Lenaerts K, Derikx JP, Matthijsen RA, de Bruine AP, van Bijnen AA, van Dam RM, Dejong CH, Buurman WA (2010) Human intestinal ischemia-reperfusion-induced inflammation characterized: experiences from a new translational model. *Am J Pathol* **176**: 2283–2291.

Gupta SK, Chong SK, Fitzgerald JF (1999) Disaccharidase activities in children: normal values and comparison based on symptoms and histologic changes. *J Pediatr Gastroenterol Nutr* **28**: 246–251.

Hedemann MS, Jensen BB, Poulsen HD (2006) Influence of dietary zinc and copper on digestive enzyme activity and intestinal morphology in weaned pigs. *J Anim Sci* **84**: 3310–3320.

Huang CC, Chen JR, Liu CC, Chen KT, Shieh MJ, Yang SC (2005) Effects of long- term ethanol consumption on jejunal lipase and disaccharidase activities in male and female rats. *World J Gastroenterol* **11**: 2603–2608.

Ikeda H, Yang CL, Tong J, Nishimaki H, Masuda K, Takeo T, Kasai K, Itoh G (2002) Rat small intestinal goblet cell kinetics in the process of restitution of surface epithelium subjected to ischemia-reperfusion injury. *Dig Dis Sci* **47**: 590–601.

Kaufman SS, Wisecarver JL, Ruby EI, Fox IJ, Sudan DL, Pillen TJ, Dhawan A, Horslen SP, Vanderhoof JA, Radio SJ, Markin RS, Langnas AN (1998) Correlation of mucosal disaccharidase activities with histology in evaluation of rejection following intestinal transplantation. *Pediatr Transplant* **2**: 134–138.

Kim YS, Ho SB (2010) Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Curr Gastroenterol Rep* **12**: 319–330.

Kjellev S (2009) Trefoil factor family – small peptides with multiple functionalites. *Cell Mol Life Sci* **66**: 1350–1369.

Kuusanmäki P, Lauronen J, Paavonen T, Pakarinen M, Savilahti E, Häyry P, Halttunen J (1996) Brush border enzymes in evaluation of acute small bowel rejection. *Scand J Immunol* **43**: 566–573.

Kuusanmäki P, Lauronen J, Paavonen T, Pakarinen M, Yilmaz S, Häyry P, Halttunen J (1997) How to diagnose chronic rejection. A study in porcine intestinal allografts. *Scand J Immunol* **46**: 514–519.

Li Y, Zhu L, Li J (2006) Two-step procedure of whole orthotopic intestinal transplantation in rats: considerations of techniques and graft functional adaptation. *Microsurgery* **26**: 399–403.

Lin CH, Correia L, Tolia K, Gesell MS, Tolia V, Lee PC, Luk GD (1998) Early weaning induces jejunal ornithine decarboxylase and cell proliferation in neonatal rats. *J Nutr* **128**: 1636–1642.

Nano JL, Cefai D, Rampal P (1990) Effect of ethanol on an intestinal epithelial cell line. *Alcohol Clin Exp Res* **14**: 32–37.

Oku T, Tanabe K, Ogawa S, Sadamori N, Nakamura S (2011) Similarity of hydrolyzing activity of human and rat small intestinal disaccharidases. *Clin Exp Gastroenterol* **4**: 155–161.

Oltean M, Pullertis R, Zhu C, Blomgren K, Hallberg EC, Olausson M (2007) Donor pretreatment with FK 506 reduces reperfusion injury and accelerates intestinal graft recovery in rats. *Surgery* **141**: 667–677.

Payne RL, Bidner TD, Fakler TM, Southern LL (2006) Growth and intestinal morphology of pigs from sows fed two zinc sources during gestation and lactation. *J Anim Sci* **84**: 2141–2149.

Perreault C, Jutras J, Roy DC, Filep JG, Brochu S (1996) Identification of an immunodominant mouse minor histocompatibility antigen (MiHA). T cell response to a single dominant MiHA causes graft-versus-host disease. *J Clin Invest* **98**: 622–628.

Pluske JR, Thompson MJ, Atwood CS, Bird PH, Williams IH, Hartmann PE (1996) Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. *Br J Nutr* **76**: 409–422.

Porter CW, Dworacyzk D, Gurtoo HL (1982) Biochemical localization of aryl hydrocarbon hydroxylase in the intestinal epithelium of the rat. *Cancer Res* **42**: 1283–1285.

Prasad KK, Thapa BR, Nain CK, Sharma AK, Singh K (2008) Brush border enzyme activities in relation to histological lesion in pediatric celiac disease. *J Gastroenterol Hepatol* **23**: e348–e352.

Quaedackers JS, Beuk RJ, Bennet L, Charlton A, oude Eqbrink MG, Gunn AJ, Heineman E (2000) An evaluation of methods for grading histologic injury following ischemia/reperfusion of the small bowel. *Transplant Proc* **32**: 1307–1310.

Raul F (1988) Age related increase of brush border enzyme activities along the small intestine. *Gut* **29**: 1557–1563.

Rodriguez-Castilla J, Lopez-Nuevo M, Delgado MJ, Murillo ML, Carreras O (1996) Changes in the ileal disaccharidases activities in the rats after long-term ethanol feeding. *Alcohol Alcohol* **31**: 69–74.

Sabat P, Veloso C (2003) Ontogenic development of intestinal disaccharidases in the precocial rodent *Octodon degus* (Octodontidae). *Comp Biochem Physiol A Mol Integr Physiol* **134**: 393–397.

Stallion A, Kou TD, Miller KA, Dahms BB, Dudgeon DL, Levine AD (2002) IL-10 is not protective in intestinal ischemia reperfusion injury. *J Surg Res* **105**: 145–152.

Su B (1998) Study progress on apoptosis and ischemia-reperfusion injury. *Foreign Med Surg* **25**: 325–327.

Taguchi T, Yamada T, Suita S, Ohta M (1995) The significance of cytological examination on reperfusion in rat small intestinal transplantation. *Virchows Arch* **427**: 71–76.

Taupin D, Podolsky DK (2003) Trefoil factors: initiators of mucosal healing. *Nat Rev Mol Cell Biol* **4**: 721–732.

Teitelbaum DH, Wise WE, Sonnino RE, Dunawy DJ, Powers P, McClung HJ, Harmel RP Jr (1989) Monitoring of intestinal transplant rejection. *Am J Surg* **157**: 318–322.

Tori AJ, Carroll AE, Gupta SK (2007) Disaccharidase activity in infants and comparison based on symptoms and histological changes. *J Pediatr Gastroenterol Nutr* **45**: 194-198.

Toth Š, Pomfy M, Wohlfahrt P, Pingorová S, Kišš J, Baláž P, Rokošný S, Beňačka R, Veselá J (2007) Detection of early stages of apoptosis in experimental intestinal ischemia-reperfusion injury. *Biologia* **62**: 491–497.

Tóth S, Jonecová Z, Varga J, Staško P, Kovalčinová B, Maretta M, Veselá J (2012) Mesenteric ischemia-reperfusion injury: Specific impact on different cell populations within the jejunal wall in rats. *Acta Histochemica* **114**: 276–284

Tran CD, Cool J, Xian CJ (2011) Dietary zinc and metallothionein on small intestinal disaccharidases activity in mice. *World J Gastroenterol* **17**: 354–360.

Varga J, Stasko P, Tóth S, Pristásová Z, Bujdos M, Pomfy M (2009) Development of jejunal graft damage during intestinal transplantation. *Ann Transplant* **14**: 62–69.

Varga J, Stasko P, Tóth S, Pristášová Z, Jonecová Z, Veselá J, Pomfy M (2010) Morphological and apoptotic changes in the intestinal mucosa and lung parenchyma after ischaemic/reperfusion injury of the jejunum. *Acta Vet Hung* **58**: 243–256.

Varga J, Tóth S, Staško P, Bujdoš M, Veselá J, Jonecová Z, Pomfy M (2011) Different ischemic preconditioning regimens affecting preservation injury of intestines. *Eur Surg Res* **46**: 207–213.

Vieira MR, Galvão LC, Fernandes MI (2000) Relation of the disaccharidases in the small intestine of the rat to the degree of experimentally induced iron-deficiency anemia. *Braz J Med Biol Res* **33**: 539–544.

Yeh KY, Yeh M, Glass J, Granger DN (2000) Rapid activation of NK-kappaB and AP-1 and target gene expression in postischemic rat intestine. *Gastroenterology* **118**: 525–534.

Yildiz Y, Kose H, Cecen S, Ergin K, Demir EM, Serter M (2010) Protective effects of leflunomide on intestinal ischemia-reperfusion injury: leflunomide against intestinal ischemia-reperfusion. *Dig Dis Sci* **55**: 245–252.

Yildiz Y, Serter M, Ek RO, Ergin K, Cecen S, Demir EM, Yenisey C (2009) Protective effect of caffeic acid phenethyl ester on intestinal ischemia-reperfusion injury. *Dig Dis Sci* **54**: 738–744.