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Intramucosal pH and intestinal mucosal damage in ischemia-reperfusion injury

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Abstract Small bowel transplantation (SBT) has become an increasingly promising treatment for short bowel syndrome. The evaluation of graft viability after SBT, however, has not been established, except by mucosal biopsy. We monitored intestinal mucosal acidity in order to detect small intestinal ischemia-reperfusion injury. Mongrel dogs were used in this study. After laparotomy, the small bowel was isolated with a vascular pedicle. A tonometer to measure intramucosal pH (pHi) was then positioned in the terminal ileum. The superior mesenteric artery was occluded with or without concomitant superior mesenteric vein occlusion for 60 or 120 min. The value of pHi was determined from laparotomy (baseline) to 12 h after reperfusion. Whole-thickness specimens of the ileum were taken before ischemia, just before reperfusion, and 1 h afterward. Mucosal injury was graded histopathologically. pHi decreased from baseline in relation to the degree of histopathological mucosal injury. There was a significant correlation between histological findings and the change in pHi. We conclude that monitoring intestinal mucosal acidity is a reliable way of determining graft viability after SBT.

Key words Intramucosal pH, ischemia-reperfusion injury · Ischemia-reperfusion injury, intramucosal pH · Small bowel preservation

Introduction

Patients with intestinal failure depend upon long-term total parenteral nutrition (TPN) for survival. Small bowel transplantation (SBT) offers these patients a potential alternative to the lifestyle restrictions, complications, and costs associated with long-term TPN [8, 17]. Unfortunately, the results of SBT have often been disappointing because of poor graft function, severe rejection, graft-versus-host disease, infection, and complications of immunosuppressive therapy [11, 15, 30, 34, 35]. Recently, long-term survival after SBT has been enhanced by combined liver and small bowel grafting [6, 18, 20] or treatment with FK506 [11, 34], bringing SBT closer to clinical practice. The assessment of graft viability after SBT and the prevention or early detection of rejection are crucial for successful clinical SBT [30, 34, 35].

The small intestine is particularly susceptible to injury from ischemia and reperfusion. Structural damage to the small intestinal mucosa is observed remarkably soon after the onset of an ischemic period. Some authors have studied the development of morphological changes in the small intestinal mucosa with increasing periods of ischemia with both light [7] and electron microscopy [5]. However, histopathological examination of mucosal punch biopsy specimens is only a partial evaluation. During ischemia and subsequent reperfusion, hypothermia and oxygen deprivation induce endothelial swelling and cause a decline in tissue pH [2]. Boros et al. [4] reported that a 120-min intestinal ischemia time caused a progressive fall in intramucosal pH

Table 1 Microscopic criteria for grading intestinal mucosal injury

Grade	Description
0	Normal mucosal villi
1	Slight elevation of epithelium from lamina propria at apex of villi
2	Moderate elevation of epithelial layer from lamina propria
3	Massive epithelial elevation extending down sides of villi
4	Denuded villi with lamina propria exposed and dilated capillaries
5	Disintegration of lamina propria; hemorrhage and ulceration

(pHi), and that reperfusion resulted in a slow return to nearly normal pHi values. However, they did not consider the relationship between histological intestinal mucosal damage and pHi. As the tonometric measurement of intestinal mucosa is readily and safely performed [10], we designed this study to clarify the correlation between histological intestinal mucosal change and pHi in intestinal ischemia-reperfusion injury.

Materials and methods

Twenty-seven healthy mongrel dogs of both sexes weighing 12–20 kg were used. With free access to water, each dog was fasted for 24 h prior to the experiment. After the administration of ketamine hydrochloride (10 mg/kg intramuscular injection), the animals were anesthetized with pentobarbital sodium (10 mg/kg) and pancuronium bromide (0.2 mg/kg), intubated, and connected to a volume-cycled ventilator (MD800, Senko Med. Co. Ltd., Tokyo, Japan) at a tidal volume of 20 ml/kg and a rate of 15 breaths/min. Positive end-expiratory pressure was controlled at 5.0 cm H_2O. Muscular relaxation was obtained with additional pancuronium bromide (0.1 mg/kg). A polyethylene catheter was positioned in the carotid artery and connected to a pressure transducer for recording of arterial pressure. Through a skin incision, a polyethylene catheter for blood sampling was passed via the right femoral vein into the right hepatic vein. The catheter was also used to infuse a lactated Ringer's solution during the experiment at the rate of 10 ml/kg per hour to compensate for fluid losses due to surgery. Electrocardiograms and esophageal temperature were continuously monitored throughout the study.

Laparotomy was performed after blood pressure and respiratory parameters had stabilized. After laparotomy via a midline incision, the small bowel was isolated with a vascular pedicle from the proximal jejunum to the terminal ileum. Both the superior mesenteric artery and vein were isolated from surrounding lymph nodes, plexuses, and tissues. A small antimesenteric incision was made in the terminal ileum, and a tonometer (Trip, Tonometrics, Helsinki) was placed in the lumen of the gut and secured with a purse-string suture. Animals were randomly placed in one of four groups for different treatments. Group 1 animals ($n = 7$) received 60-min superior mesenteric artery (SMA) occlusion. Group 2 animals ($n = 6$) received 120-min SMA occlusion, and group 3 animals ($n = 8$) received both SMA and SMV occlusion for 120 min.

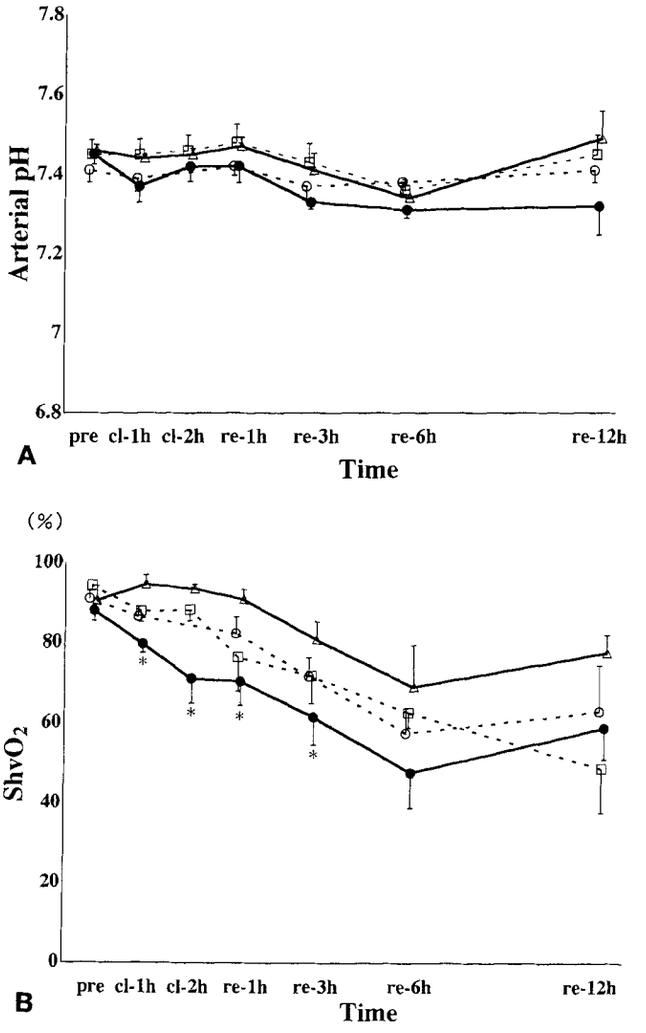

Fig. 1 A, B Parameters used to calculate intramucosal pH over time: **A** Arterial pH. Group 1, open circles, broken line; group 2, open squares, broken line; group 3, filled circles, solid line; group 4, open triangles, solid line. Values are expressed as the mean ± the SEM; **B** Hepatic venous oxygen saturation ($ShvO_2$). (*Pre* preischemia, *cl* clamping, *re* reperfusion) * $P < 0.05$ compared to group 4

Group 4 animals ($n = 6$) formed a control in which a sham operation was performed for a comparable time under the same anesthesia.

Mean arterial pressure was measured continuously with transducers. An arterial blood sample was taken via the arterial catheter, and arterial pH was determined at the time of laparotomy, during ischemia, and 1, 3, 6, and 12 h after reperfusion. A hepatic venous blood sample was taken simultaneously and analyzed for oxygen saturation ($ShvO_2$) using a blood gas analyzer (ABL520; Radiometer, Copenhagen, Denmark). pHi was calculated using the method of Fiddian-Green [13]. The method was based on the principle that PCO_2 in the fluid within a semipermeable balloon attached to a catheter equilibrated with that in the lumen of a hollow

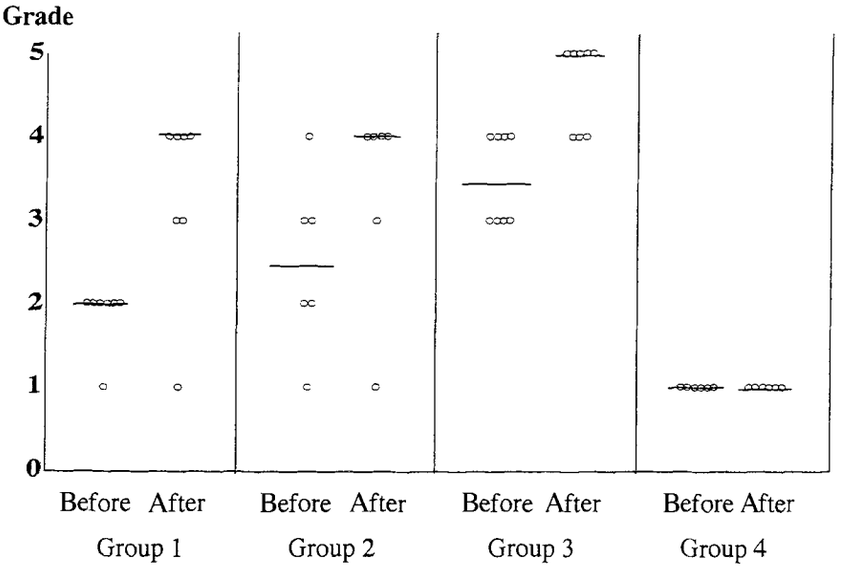

Fig. 2 Mucosal injury grade according to Chiu et al.'s classification [7] just before and after 1 h of reperfusion in each group. Each symbol represents one experimental animal, and the lines represent the median value for each group

organ and in turn, with that in its mucosa. If the arterial HCO_3^- concentration is known, the pHi can be calculated. The HCO_3^- concentration in the wall of the gut was assumed to be the same as that in arterial blood [19]. The balloon of the tonometer, placed in the terminal ileum, was filled with 2.5 ml saline via the rubber tube. After 40 min of equilibration through the wall of the balloon, the first 1.5 ml of the saline was aspirated and discharged, this volume representing the dead space of the tube. The remaining 1 ml was then immediately aspirated, and the PCO_2 of the aliquot was determined using the same type of blood gas analyzer referred to earlier. pHi was then calculated using the luminal PCO_2 and the arterial bicarbonate concentration to represent the intramucosal values in the Henderson-Hasselbalch equation, $pHi = 6.1 + \log_{10}[(\text{arterial } HCO_3^-)/(0.03 \times \text{saline } PCO_2)]$ [14]. The value of pHi before clamping was stipulated as the baseline value. pHi was then measured at 1, 3, and 12 h after reperfusion. Finally, the change in pHi from the baseline was calculated.

Whole-thickness specimens for histopathological study were obtained from the ileum before ischemia, just before reperfusion, and 1 h after reperfusion. Sections were routinely processed and stained with hematoxylin and eosin before examination by one pathologist who was unaware of animal group and time of tissue sampling. Pathological mucosal injury was graded from 0 to 5 according to a scale described by Chiu et al. [7] (Table 1). Grade 0 was defined as normal mucosa, with grades 1–5 indicating increasing degrees of villous damage.

The experimental animals were sacrificed by an overdose of pentobarbital at 12 h after reperfusion. All animals were cared for in accordance with the guidelines set forth by the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 86-23, revised 1985).

All data were expressed as means ± standard errors of the mean (SEM). Differences between means were analyzed using the Fisher test with an analysis of variance (ANOVA). P values less than 0.05 were considered significant. In establishing the correlation between the degree of mucosal injury and the change in pHi, Spearman's rank correlation coefficient and the corresponding P-value were calculated.

Results

Mean arterial pH and $ShvO_2$ values are shown in Fig. 1 A and 1 B, respectively. In group 3, a small decline in arterial pH was observed with no significant difference among experimental groups (Fig. 1 A). In groups 1, 2, and 3, $ShvO_2$ gradually decreased after vascular occlusion. The maximal decrease in $ShvO_2$ in group 2 reached 48.8% after 12 h of reperfusion. In group 3, the maximal decrease in $ShvO_2$ after 6 h of reperfusion was 47.6%. There were no significant differences in $ShvO_2$ between control animals and SMA occlusion animals. $ShvO_2$ in group 3, however, was significantly less than in controls after 1 and 2 h of vascular occlusion and after 1 and 3 h of reperfusion (Fig. 1 B).

Figure 2 shows the grade of mucosal injury according to Chiu et al.'s classification just before and after 1 h of reperfusion in each group. In all groups, the first specimens taken shortly after laparotomy were graded 0–1. In groups 1 and 2, the histological severity of mucosal injury was similar. The mucosal changes just before reperfusion showed a moderate elevation in the epithelium away from the lamina propria and areas of marked epithelial elevation over the sides of villi. The median histological grade was 2 in group 1 (range 1–2) and 2.5 in group 2 (range 1–4). After 1 h of reperfusion, villi were seen to be denuded of tissue superficial to the lamina propria (Fig. 3 A); the median grade was 4 in groups 1 and 2 (range 1–4). In group 3, the median grade was 3.5 (range 3–4) just before reperfusion. After 1 h of reperfusion, further injury was demonstrated with total destruction of the villous layer and partial injury to the crypts (Fig. 3 B), resulting in a median grade of 5 (range 4–5). In group 4, all specimens showed slight

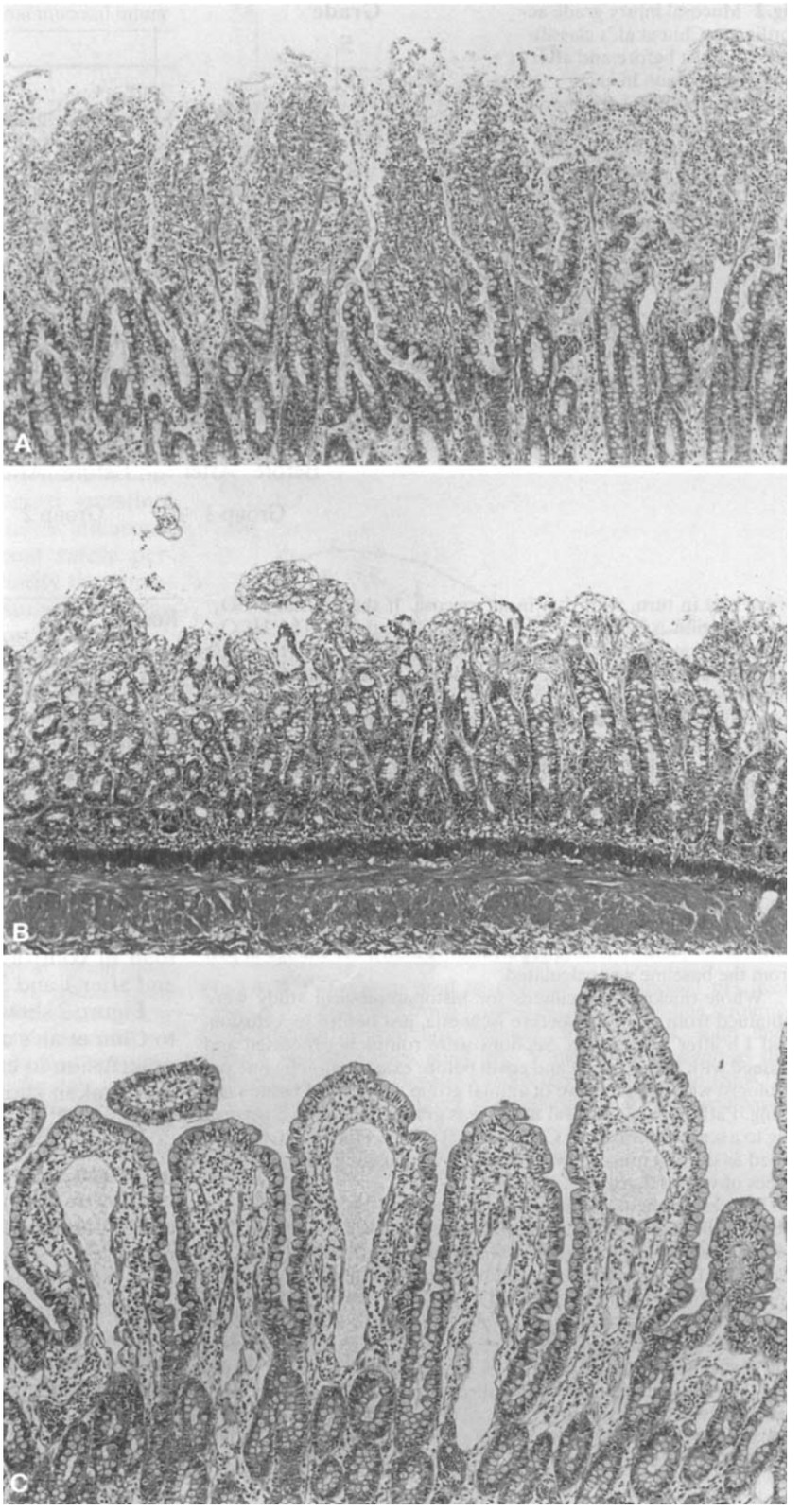

Fig. 3 A–C Intestinal mucosal appearances after 1 h of reperfusion: **A** group 2; **B** group 3; **C** group 4 (All hematoxylin and eosin, × 250)

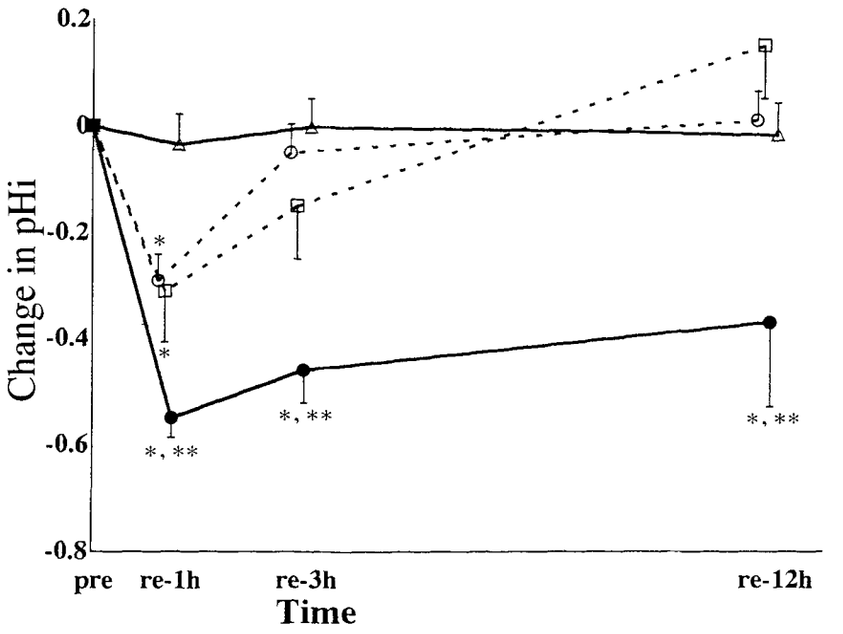

Fig. 4 Change in pHi from baseline over time (*Pre* preischemia, *cl* clamping, *re* reperfusion) * $P < 0.05$ compared to group 4; ** $P < 0.05$ compared to groups 1 and 2

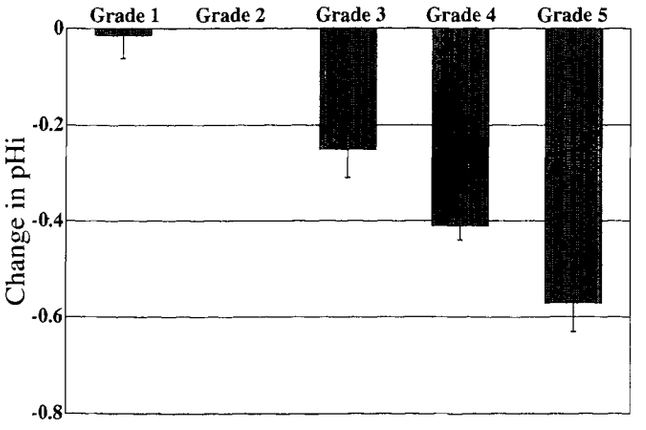

Fig. 5 Histological damage to intestinal mucosa 1 h after reperfusion and change in pHi from baseline. The decline in pHi from the baseline was correlated to the histopathological degree of mucosal injury ($r_S = -0.908$; $t = -10.665$; $df = 24$; $P < 0.001$)

epithelial elevation above apices of villi (grade 1 injury, Fig. 3C).

Changes in pHi from baseline levels are plotted in Fig. 4. In groups 1 and 2, the maximal fall in pHi was observed 1 h after reperfusion (0.29 ± 0.05 in group 1 and 0.31 ± 0.09 in group 2). This decrease was significantly greater than that with the sham-operated controls (group 4). In these groups, the pHi value had nearly returned to baseline by 12 h after reperfusion. In group 3, pHi decreased to 0.55 ± 0.03 after 1 h of reperfusion; this was a significant difference compared with groups 1 and 2. In this group there was no recovery of pHi within 12 h. Figure 5 shows the correlation between histological damage of the intestinal mucosa and changes in pHi from the baseline value to 1 h after reperfusion. Spearman's rank correlation coefficient and the corresponding P value were calculated, and the decline in pHi from the baseline was correlated to the histopathological degree of mucosal injury ($r_S = -0.908$; $t = -10.665$; $df = 24$; $P < 0.001$).

Discussion

SBT is being attempted in patients with intestinal failure in order to allow them to resume a more normal lifestyle than that permitted by TPN. However, the transplanted intestine frequently sustains a variety of ischemic injuries during preservation and after reperfusion. Hypoxia and subsequent reoxygenation are generally considered to be major factors mediating tissue destruction. Several studies have demonstrated that the adverse reaction is not usually initiated by hypoxia, but rather by the return of oxygenated blood to ischemic tissues [16, 25]. The production of oxygen-derived free radicals during reperfusion causes further cell damage and induces much injury to small intestinal mucosa [29]. The mucosa is particularly sensitive to ischemia and reperfusion, with visible damage developing rapidly after the onset of an ischemic episode. Prolonged severe ischemia results in mucosal damage, sloughing, edema, bleeding, and ulceration. Animal studies have shown that these changes begin at the tips of mucosal folds and villi.

Yet, the small intestine also possesses remarkable regenerative potential. Robinson et al. [26] reported the recovery of intestinal mucosa after ischemia to be remarkably rapid: 1 day after 1-h ischemia, the villi of canine ileum were once again covered with nearly normal epithelium. Park et al. [24] demonstrated a reperfusion component of tissue injury following complete ischemia only when no concomitant venous congestion was present, and the ischemic injury was not too extensive. They proposed that intense tissue injury caused by more severe forms of ischemia itself might result in failure to demonstrate a discernible reperfusion component.

In a clinical setting, the microscopic examination of intestinal mucosal specimens is considered a sensitive and specific method for studying damage following ischemia and reperfusion. Graft viability after SBT is assessed mainly histopathologically; however, the punch biopsy method is limited by a sampling error. Alternative techniques used to estimate intestinal graft viability include absorption [3, 9, 12, 22] and motility studies [27, 28, 32] and measurements of brush-border enzymes or Na^+-K^+ ATPase activity [33]. These approaches, however, require significant time or repeated biopsy and assay, and not only do they put the graft at risk, but they are unpleasant for the patient. On the other hand, small bowel tonometry is rapid, inexpensive, and can be performed anywhere a blood gas analyzer is available. pHi is a commonly used marker of metabolic state and blood flow in the gastrointestinal mucosa. Antonsson et al. [1] reported comparable prolongation of intramucosal acidosis detected tonometrically following complete SMA occlusion in pigs. Deardon et al. [10] reported that pHi correlated with reperfusion injury, pelvic sepsis, and graft infarction in an experimental situation.

In our experimental study, intestinal ischemia and reperfusion did not induce a significant drop in arterial pH among the experimental groups. $ShvO_2$ represents the sum of hemoglobin oxygen saturation in the blood at the venous ends of all sinusoids in the liver, and its value reflects the oxygen supply-demand relation in the liver [31]. Our data suggested that combined SMA and SMV occlusion for 120 min induced hepatic hypoperfusion. In this study, intestinal ischemia and reperfusion induced a drop in pHi from the baseline, and a progressive decrease in mucosal pHi was demonstrated, particularly following combined SMA and SMV occlusion. Histopathologically, the complete occlusion of the SMA or of both the SMA and SMV for 1–2 h, followed by reperfusion, produced extensive mucosal lesions of the entire small intestine, with disintegration of the lamina propria. With simple SMA occlusion, mucosal injury involved epithelial elevation and denuded villi. With combined SMA and SMV occlusion, mucosal injury included disintegration of the lamina propria and injury to the crypts, making mucosal injury of combined SMA and SMV occlusion much more severe than that of simple SMA occlusion.

Park et al. [23] evaluated mucosal injury during ischemia or at reperfusion microscopically using a grading scale in a model of SBT without cold ischemia, with 5 h of cold ischemia, and with 18 h of cold ischemia in the rat. They suggested that morphological injury to the intestinal mucosa might reflect graft viability after SBT. In contrast, Montgomery et al. [21] reported that the tonometer technique could be used to study mucosal microcirculation after cold storage and reperfusion of a transplanted intestine using a porcine pancreaticoduodenal allograft model.

In our experimental study, the further the pHi value fell, the more severe the mucosal morphological injury appeared, and the decline in pHi from baseline values was related to the histopathological degree of mucosal injury. Thus, tonometric pHi monitoring appears to be well suited to the estimation of graft viability after SBT. Further investigations are needed using the SBT model and clinical trials may be considered.

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