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Protective effect of ischemic preconditioning on the jejunal graft mucosa injury during cold preservation

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ABSTRACT

Protection of intestinal graft mucosa during cold preservation is still an unmet need in clinical practice, thus affecting the success of transplantation. The present study investigates the ability of two ischemic preconditioning (IPC) procedures to limit cold preservation injury. Three groups of Sprague–Dawley rats were recruited (n = 11 each) as follows: the short IPC (SIPC) performed through 4 cycles of mesenteric ischemia of 4 min each followed by 10 min of reperfusion, the long IPC (LIPC) obtained by 2 ischemic cycles of 12 min each followed by 10 min of reperfusion, and the control group (C) without IPC. Grafts were then stored in cold histidine–tryptophan–ketoglutarate solution and samples were taken at 0, 3, 6 and 9 h lasting preservation. Both IPC groups showed an advanced degree of preservation with delayed development of graft mucosa damage, mainly in the crypt region. At the beginning of preservation, the graft mucosa in both IPC groups showed lower degree of mucosal injury index (MII) by 50% in comparison with C group. Specifically, a significant improvement of MII was observed after 3 h of preservation in the LIPC group ($p < 0.05$) in comparison with untreated C grafts. Significant atrophy of the intestinal mucosa in C group was found after 3 h of preservation ($p < 0.01$), in SIPC group the progress of atrophy was delayed to 6 h ($p < 0.001$), and in LIPC group only moderate decrease in that was found. A parallel increase of laminin expression with the MII rate after 6 and 9 h of preservation in comparison with the level at time 0 was observed in all grafts ($p < 0.001$ and $p < 0.01$, respectively). In both IPC groups the apoptotic cell (AC) rate was significantly reduced at the beginning of cold preservation ($p < 0.05$ both). Moreover, in both the SIPC and C groups, the progressive increase in MII rate connected with AC rate decrease was due to a predominance of necrosis. By contrast in the LIPC group, after an increase of nearly 50% in the AC rate at the 3rd hour, its level remained fairly constant during the further 6 h of preservation, thus probably preventing necrosis and improving graft viability.

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1. Introduction

The peculiar metabolic properties and the complex network between mucosal architecture and its function in the small intestine (SI) have faced many challenges to achieve successful preservation for a successful transplantation. Specifically, the loss of mucosal barrier integrity with subsequent bacterial translocation, and the dramatic increase in inflammation lead to SI injury during hypothermic storage, which in turn results in an increased risk of life-threatening infection (McKeehen et al., 2010). Intestinal graft cold preservation is based on an initial vascular perfusion followed by static cold storage in a specific solution and is significantly shorter than in the case of solid organs such as the liver or the kidney. The current clinical approach allows cold preservation for less than 10 h because of subsequent mucosal barrier disruption (Oltean et al., 2015). The SI is a highly perfused organ, receiving about 25% of blood volume at each heartbeat of which 90% is consumed by the mucosa and submucosa, thus being the areas early affected by ischemia (Maathuis et al., 2007; Oltean et al., 2010). Cold preservation injury is caused primarily by ischemia and hypothermia, and the consequent changes depend on the duration of preservation. Within minutes from the ischemia onset, the histological changes begin

Abbreviations: AC, apoptotic cell; BM, basement membrane; C, control group; IPC, ischemic preconditioning; IR, ischemia/reperfusion; LIPC, long IPC; LDH, lactate dehydrogenase; MII, mucosal injury index; SIPC, short IPC; SI, small intestine.

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at the mucosal level, the layer most sensitive to hypoxic insult (Salehi et al., 2003). With increasing storage duration (4–6 h), progressive damage and eventual loss of the intestinal mucosa occur, resulting in failure of barrier function and absorptive capacity (6–9 h; Grant et al., 1991). IPC is an effective surgical technique that increases the tolerance of the SI to the ischemic insult of manipulation and preservation, therefore protecting the organ from ischemia/reperfusion (IR) injury (Mallick et al., 2004). It refers to the strategy of an initial brief period of ischemia (Mallick et al., 2005) whose effects can be differentiated into 2 phases: (1) early or classic preconditioning (first window of protection), which immediately follows the transient ischemia and lasts 2 to 3 h; and (2) late preconditioning (second window of protection), which begins 10 to 24 h from the transient ischemia and lasts for about 3 to 4 days (Pasupathy and Homer-Vanniasinkam, 2005). The protective effect of one cycle ischemia (10 min) followed by reperfusia (5–30 min) prior to graft harvesting has been confirmed in several publications. After transplantation in preconditioned grafts was found significantly lower tissue injury, smaller lesions of basement membrane (BM), inhibition of epithelial cell apoptosis, decrease in serum lactate dehydrogenase (LDH), aspartate aminotransferase and alanine transaminase and significantly increased heme oxygenase as an indicator of protection against IR injury (Kang et al., 2014; Saeki et al., 2011; Sola et al., 2001; Wang and Li, 2003), graft IPC prevented inflammatory response in recipients after intestinal transplantation, measured by plasma LDH and neutrophil recruitment in the intestine. They also found lower histopathological injury in IPC grafts after 4 h of cold preservation and lower LDH release into preservation solution as a marker of tissue injury. Our previous study (Varga et al., 2011) showed a protective effect of IPC against graft injury arising during cold storage, which was affected by different IPC regimens. Briefly, a higher graft protection was observed when fewer, but longer periods of ischemic insult were used in IPC, thanks to the improved morphology of the SI wall and the integrity of goblet cell population within the graft epithelium during the preservation period. Thus the first objective of present study was to assess histopathological alterations and morphometric measure of the SI mucosa since it is the most sensitive and vulnerable site to the preservation injury. Specifically, the aims of our work were to compare the effect of two different IPC procedures on the graft preservation and to evaluate histological injury and morphometric modification of SI mucosa in relation to duration of hypothermic ischemia.

Under physiological conditions SI mucosa shows a remarkable turnover, and a rapid regeneration of the epithelial layer has been reported after intestinal IR (Tóth et al., 2012). Studies on wound healing have revealed that laminin-5 plays a key role in the healing processes and it also plays an important role in the ordered migration and differentiation process of enterocytes along the crypt/villus axis (Gout et al., 2001; Leivo et al., 1996). Laminins are major components of the BM mediating cell adhesion and generating differentiation signals when bound to their surface receptors. Zuk and Matlin (2002) demonstrated a significantly increased expression of laminin-5 and laminin receptor α_3 integrin subunit in the BM of ischemic kidney, which may indicate a role for this receptor–ligand interaction in the pathogenesis of acute renal failure and/or repair of the injured kidney epithelium. According to Wang and Li (2003) one of the mechanisms of IPC could be the inhibition of epithelial cell apoptosis. Thus the second objective of our study was to investigate the effect of different intervals of cold storage and IPC on epithelial apoptosis and laminin expression because of their importance in preserving barrier function. Determination of the optimal IPC design with the strongest protective effect still remains to be solved in experimental studies.

2. Materials and methods

This study was approved by the Ethics Committee on Animal Experiments of the Faculty of Medicine, Pavol Jozef Safarik University, Kosice, Slovak Republic, and the experimental protocol was approved by the State Veterinary and Food Administration of the Slovak Republic (No. 2843/08-221a).

2.1. Experimental design

Male Sprague–Dawley rats (288 ± 50 g) were housed in standard conditions with controlled temperature (21 °C) and had access to commercial chow and water *ad libitum*. The animals were randomly divided into 3 groups (n = 11 each): controls (group C); animals receiving a short IPC procedure (group SIPC), and those receiving a long IPC procedure (group LIPC). Selected IPC procedures were chosen according to our previous experimental study (Varga et al., 2011).

2.2. Surgical procedures and sampling

Animals were fasted overnight in cages with raised floors to minimize coprophagy. Water was provided *ad libitum*. Total anaesthesia was induced by intraperitoneal injection of a mixture of ketamine 10 mg/100 g (Narketan 10%, Vétoquinol) and xylazine 1.5 mg/100 g (Xylariem 5%, Riemser). After 1 h, 1/3 of the total dose of the anaesthetic mixture was added intramuscularly. Body temperature was maintained by a heating pad set to 37 °C until the end of the operation. In the untreated group (C), harvest and preservation of unpreconditioned grafts were performed after midline laparotomy. In groups SIPC and LIPC, intestines were preconditioned with different IPC procedures, as follows: through 4 cycles of mesenteric ischemia of 4 min each followed by 10 min of reperfusion (short IPC, SIPC) or 2 ischemic cycles of 12 min each followed by 10 min of reperfusion (long IPC, LIPC). After application of IPC schedules in the pretreated groups, intestines were harvested. Thereafter, unpreconditioned (C) as well as preconditioned grafts (SIPC and LIPC) were stored at 4 °C in Custodiol HTK (histidine–tryptophan–ketoglutarate) preservation solution for 9 h. Biopsies were taken at 0, 3, 6 and 9 h of preservation (samples T0, T3, T6 and T9), and the obtained samples were immediately rinsed in cold saline and processed for routine histological analysis.

2.3. Histological analysis

Routine haematoxylin–eosin staining method was used for evaluation of jejunal mucosa damage according to our scoring system published elsewhere (Kovalčinová et al., 2014). MII was graded as follows: 0 — normal (no pathological defects), 1 — intestinal villi epithelia damage (swelling, vacuolation, progressive epithelial lifting from BM particularly at the villus tip), 2 — intestinal villi epithelia and lamina propria damage (local necrosis of the epithelium, apical villi denudation, dystrophy of connective tissue in the villi, capillary dilatation, epithelial detachment from BM at the villus base), 3 — damage of intestinal crypt epithelial lining and lamina propria (massive denudation with necrotic lesions and haemorrhages in middle region of the villi), 4 — complete destruction of mucosa with damage to *lamina muscularis mucosae* (absence of villus tissue, distinct basophilia and dystrophy of intestinal crypt epithelium, capillary dilatation and congestion, necrotic lesions and haemorrhages in connective tissue of crypt regions, discontinuity of *lamina muscularis mucosae*).

2.4. Morphometrical analysis

Morphometry was performed using an Olympus BX50 light microscope with Olympus Camera SP350 (Olympus, Tokyo, Japan) and QuickPHOTO Industrial 2.3 image analyser software (Promicra, Prague, Czech Republic). Mucosa thickness was assessed by measuring the distance between the villus tip to the *lamina muscularis mucosae* in 12 axially-oriented villi in at least three different quadrants of each intestinal sample. Cell number in the *lamina epithelialis mucosae* was calculated *per* unit of mucosal epithelial lining (mm) or *per* villus. All measurements were done using magnification 200 × .

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