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Luminal solutions protect mucosal barrier during extended preservation

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ABSTRACT

Background: Mucosal barrier injury during intestinal preservation (IP) and transplantation favors life-threatening infections. Luminal delivery of solutions containing amino acids or polyethylene glycols (PEGs) may improve preservation results and reduce this injury. We tested if solutions containing glutamine and PEG influence the mucosal injury.

Materials and methods: Rat intestines were perfused and stored in Viaspan-University of Wisconsin solution. Before IP, a PEG 3350 solution was introduced intraluminally alone (group 1) or supplemented with 40 mmol/L L-glutamine (group 2). Controls underwent vascular flush alone (group 3). Preservation injury was evaluated after 8, 14, and 24 h by histology and goblet cell count. Tight-junction proteins zonula occludens-1, claudin-3, claudin-4, and caveolin-1 were studied by immunofluorescence. Maltase and caspase-3 activity were also analyzed.

Results: Group 1 showed mild edema at 8 h and mucosal disruption by 24 h; these features were greatly improved in group 2 where continuous mucosa was found after 24 h of IP. Intestines in group 3 did worse at all time points with subepithelial edema (Park/Chiu grade 3) and marked goblet cell depletion; caspase-3 activity was lowest in group 2. Tight-junction proteins varied continuously during IP; zonula occludens-1 expression and colocalization with claudins decreased significantly in group 3 but not in other groups. Claudin-3 was distinctly localized in the membrane, but stained diffuse, cytoplasmic at later time-points. Claudin-4 changed to a cytoplasmic granular pattern. No caveolin-1 colocalization was observed.

Conclusions: Luminal PEG and glutamine delay epithelial breakdown and preserve several important mucosal features during extended IP.

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1. Introduction

Intestinal transplantation recorded major advancements over the past decade because of improved patient selection, novel surgical techniques, refined immunosuppression, and monitoring [1]. Nevertheless, intestinal preservation (IP) remained unchanged despite suboptimal results and is still based on an initial vascular perfusion followed by static cold storage in University of Wisconsin (UW)–Viaspan (Bristol Myers Squibb, Solna, Sweden) or histidine-tryptophan-ketoglutarate solutions [2,3]. The current clinical approach allows for <10 h of cold preservation, significantly shorter than in the case of kidneys or liver. This relatively short time-interval in combination with the spectrum of early life-threatening septic complications secondary to the loss of the mucosal barrier remain important limiting factors in intestinal transplantation.

IP injury evolves as a progressive subepithelial edema leading to the loss of the mucosal barrier. Its development is consecutive to the dysfunction and disassembly of the energy dependent tight junctions (TJs) and leads to an increased epithelial permeability [4,5]. Experimental administration of different solutions through the luminal route has been shown effective in reducing preservation injury through several mechanisms including improved energy status or delaying the disassembly of the TJ [6]. Our group recently found that a solution containing a low molecular weight polyethylene glycol (PEG) delivered luminally before cold storage improves IP compared with vascular flush alone, an effect than seems to be related to a maintained TJ structure [7].

Glutamine is a conditionally essential amino acid that provides a major energy substrate for enterocytes, and serves as an important nitrogen source during protein metabolism. Besides these traditional functions, recent studies indicate that glutamine also acts as a messenger in several signaling pathways and modulates numerous cellular processes including cytokine production, cell proliferation, and cell viability or apoptosis. Importantly, glutamine alone or together with other amino acids decreased the IP injury when added to the vascular or luminal preservation solutions [8,9]. The protective effect of glutamine during IP, alone or as an additive in complex solutions, has been signaled previously but as to our knowledge never before been tested together with PEG [10–12]. The protective mechanism may differ between the two compounds, thus we set out to investigate any synergistic effects when glutamine and PEG are combined.

However, in the setting of IP, glutamine has mostly been studied either alone in various concentrations or in very complex solutions, none in combination with PEG. We found that this combination protects several components of the intestinal innate immunity from the injury induced by the prolonged cold storage.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (230–250 g) were purchased from B&K Universal (Sollentuna, Sweden) and acclimatized for 1 wk at the university animal facility. The animals were housed in controlled temperature and pathogen free environment, in 12 h light–dark cycles, and receiving rat chow and water *ad libitum*. The animals were not fasted before surgery and all procedures followed the regulations outlined by National Institutes of Health and the European Union and was approved by the local committee of the Swedish Animal Welfare Agency.

2.2. Surgery and sampling

The abdominal cavity was opened through a midline incision, and the portal vein was dissected free by ligating all its tributaries vessels, including pancreaticoduodenal, splenic, and pyloric vein. The middle colic vessels were ligated and divided, and the intestinal graft was fashioned from the proximal two-thirds of the small intestine. The abdominal aorta was dissected around the mesenteric artery, up to the celiac trunk and down to the right renal vessels as well as proximal to its bifurcation. Thereafter, aorta was ligated above the celiac trunk and the intestine was perfused *in situ* retrogradely through the distal aorta with 5 mL/min ice-cold UW solution until completely blood-free (8–10 mL UW in total). The right atrium was cut to facilitate venous venting. After perfusion, the graft was excised and placed in a Petri dish containing chilled UW solution. Intestines were randomly assigned to receive intraluminal PEG solution with or without supplementary glutamine. Graft ends were tightly ligated with 3-0 silk ligature, and the intestines were stored in 80 mL ice-chilled perfusion solution.

After 8, 14, and 24 h of cold storage, the intestinal segments were removed from the ice-cold solution and carefully blotted dry on a paper towel to remove the solution on the bowel surface. The intestine was then opened, and the intraluminal solution was recovered. Graft segments were either placed in 4% buffered formalin or snap-frozen ($n = 8$ per time-point).

2.3. Solutions and experimental groups

UW solution (Viaspan) was used for the intestinal perfusion and storage. For the intraluminal preservation, we used a commercially available bowel preparation solution (Movicol; Norgine, Harefield, United Kingdom) containing 5, 4 mmol/L potassium, 65 mmol/L sodium, 53 mmol/L chloride, 15 mmol/L bicarbonate, and 13.12% PEG 3350. Additional L-glutamine was added in a concentration of 40 mM in one group.

Group 1 received luminal PEG solution, group 2 received PEG solution supplemented with glutamine, whereas group 3 underwent only the vascular flush ($n = 8$ per group).

2.4. Light microscopy

Full thickness samples were formalin fixed, paraffin embedded, cut in 5 μ slides and stained with hematoxylin-eosin. At least six sections at three different levels were examined. The ischemic injury was scored in a blinded fashion using Park score [13].