Optimal Solution Volume for Luminal Preservation: A Preclinical Study in Porcine Intestinal Preservation

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ABSTRACT

Background. Rodent studies suggest that luminal solutions alleviate the mucosal injury and prolong intestinal preservation but concerns exist that excessive volumes of luminal fluid may promote tissue edema. Differences in size, structure, and metabolism between rats and humans require studies in large animals before clinical use.

Methods. Intestinal procurement was performed in 7 pigs. After perfusion with histidine-tryptophan-ketoglutarate (HTK), 40-cm-long segments were cut and filled with 13.5% polyethylene glycol (PEG) 3350 solution as follows: V0 (controls, none), V1 (0.5 mL/cm), V2 (1 mL/cm), V3 (1.5 mL/cm), and V4 (2 mL/cm). Tissue and luminal solutions were sampled after 8, 14, and 24 hours of cold storage (CS). Preservation injury (Chiu score), the apical membrane (ZO-1, brush-border maltase activity), and the electrolyte content in the luminal solution were studied.

Results. In control intestines, 8-hour CS in HTK solution resulted in minimal mucosal changes (grade 1) that progressed to significant subepithelial edema (grade 3) by 24 hours. During this time, a gradual loss in ZO-1 was recorded, whereas maltase activity remained unaltered. Moreover, variable degrees of submucosal edema were observed. Luminal introduction of high volumes (2 mL/mL) of PEG solution accelerated the development of the subepithelial edema and submucosal edema, leading to worse histology. However, ZO-1 was preserved better over time than in control intestines (no luminal solution). Maltase activity was reduced in intestines receiving luminal preservation. Luminal sodium content decreased in time and did not differ between groups.

Conclusions. This PEG solution protects the apical membrane and the tight-junction proteins but may favor water absorption and tissue (submucosal) edema, and luminal volumes >2 mL/cm may result in worse intestinal morphology.

INTESTINAL preservation (IP) with the use of using either University of Wisconsin–Viaspan or histidine-tryptophan-ketoglutarate (HTK) results in significant mucosal barrier injury if the cold storage (CS) is extended beyond 10 hours [1]. The mucosal injury further aggravates on reperfusion and may result in postreperfusion syndrome, sepsis bouts as a result of bacterial translocation, and fluid unbalance [2,3]. Additional luminal delivery of various solutions has been suggested to delay the development of preservation injury [4,5]. However, luminal fluids may increase the risk of tissue edema as the result of paracellular water absorption through dysfunctional tight junctions (TJ). Impermeants and macromolecules with high osmotic pressure are usually added to the luminal solutions

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counteract this phenomenon. Nonetheless, the volume of luminal preservation fluid may also have some importance, but this variable has not yet been studied. Therefore, we evaluated the protective effects of various luminal volumes of a customized solution containing a low-molecular-weight polyethylene glycol (PEG) during intestinal cold ischemia in a preclinical, porcine model.

METHODS

Animals and Experimental Procedure

Landrace pigs (n = 7) weighing 25 to 35 kg were fasted 24 hours and pre-medicated with Ketamine (20 mg/kg), Xylazine (2 mg/kg), and atropine (0.05 mg/kg) then intubated and ventilated using a mixture of isoflurane (0.6% to 2%) and oxygen. After median laparotomy, a total colectomy was performed and the portal vein and the superior mesenteric artery (SMA) were identified and dissected free. The aorta was dissected circumferentially above the emergence of SMA and above the bifurcation and secured with umbilical tape. After donor heparinization (100 U/kg) and aortic cross-clamping, in situ retrograde vascular perfusion was performed with the use of 1.5 L ice-cold histidine-tryptophan-ketoglutarate solution (Custodiol, Koehler Chemie, Alsbach-Hähnlein, Germany). Thereafter, the entire intestine was removed and placed in ice-cold preservation solution. Forty–centimeter-long segments were stapled from the terminal ileum and filled with different volumes of a macromolecular solution containing 5.4 mmol/L potassium, 65 mEq/L sodium, 53 mmol/L chloride, 15 mmol/L bicarbonate, and 13.12% PEG 3350 (Movicol, Norgine, Harefield, United Kingdom) as follows: 0.5 mL/cm (V1), 1 mL/cm (V2), 1.5 mL/cm (V3), and 1.5 mL/cm (V4) or left empty as clinical controls (V0). Tissue and luminal solution were sampled after 8, 14, and 24 hours of IP. The electrolyte content in the solution was analyzed with the use of a Modular P analyzer (Roche Diagnostics, Mannheim, Germany).

Histology

Full-thickness samples were formalin-fixed, paraffin-embedded, cut into 5-μm slides, and stained with hematoxylin-eosin. The ischemic injury was graded on 7 different fields on at least 3 different sections at medium-power magnification (×10), using the Chiu score [6]. Immunofluorescence was used to study the tight-junction protein zonula occludens-1 (ZO-1). After deparaffinization, rehydration, and antigen retrieval, slides were incubated overnight at 4°C with antibodies against ZO-1 (1:100, Invitrogen, Stockholm, Sweden). After incubation with secondary antibody and counterstaining with 4′6′-diamidino-2-phenylindole, slides were examined under fluorescence microscope.

Tissue Analyses

Maltase-isomaltase activity was analyzed in whole-tissue homogenate as described elsewhere [6]. Tissue water content (wet weight/dry weight ratio) was calculated after freeze-drying for 48 hours to quantify tissue edema.

Statistical Analyses

Statistical differences between groups were analyzed by use of the Kruskal-Wallis test followed by the Mann-Whitney U test (GraphPrisma5). Results are expressed as mean ± standard deviation unless otherwise stated. A value of $P < .05$ was considered significant.

RESULTS

Histology

Eight hours of IP led to minimal morphological changes in the intestines receiving vascular perfusion alone (clinical controls), which were classed as grade 1 (subepithelial clefts). Fourteen hours of IP led to increased detachment from the lamina propria (grade 2–3), whereas 24 hours of storage generated a uniform and sizeable epithelial detachment (grade 3).

The intestines receiving between 0.5 and 1.5 mL/cm of the luminal macromolecular solution presented a trend toward preserved histology compared with the clinical controls after 8 and 14 hours, albeit the difference did not reach statistical significance. Intestines from group V4 (2 mL/cm) appeared to have less preserved morphology than control tissue.

Immunohistochemistry

In normal intestines, ZO-1 (green) was present at the contact between the basolateral and the apical membrane as a continuous, thin staining lining the luminal surface, including the crypts.

Eight hours of IP did not significantly alter significantly ZO-1 expression in intestines undergoing vascular perfusion alone (clinical controls). Fourteen hours of IP caused frequent loss of ZO-1 staining at the villus tips, and, by 24 hours of IP, a ZO-1 staining loss was evident on the enterocytes on the luminal half of the villi.

Normal or near-normal ZO-1 staining pattern was found in the intestines undergoing luminal preservation for 14 hours, irrespective of the luminal volume introduced. Prolonging the cold preservation time to 24 hours led to variable degrees of ZO-1 expression loss toward the tips of the villi, regardless of the luminal volume of solution.

Tissue Analysis

Tissue water content was always higher in the intestines receiving standard IP with vascular perfusion alone (clinical controls). In contrast, the water content in the intestines receiving luminal solutions appeared to remain constant and was lower than in the clinical controls irrespective of the luminal volume used (Fig 1B). Maltase-isomaltase activity did not vary significantly within each group and was found at similar levels in the groups undergoing luminal preservation. Interestingly, there was a trend toward higher maltase-isomaltase activity in the intestines undergoing IP without luminal preservation (Fig 1C).

Composition of Luminal Solutions

The electrolyte composition in the luminal fluid changed significantly during the IP. However, sodium content varied similarly in all 4 groups and slowly decreased over the tie (Fig 1D). Potassium increased rapidly and appeared to stabilize at approximately 12 mEq. The increase was slower with increasing luminal solution volume, probably because of the dilution in the higher volume of liquid (not shown).