Experimental small bowel preservation using Polysol

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Zusammenfassung

Die hohe Anfälligkeit des Dünndarmes in Bezug auf den Konservierungs- und Reperfusionsschaden stellt immer noch ein Hindernis für eine erfolgreiche Transplantation dar. In der gegenwärtigen Studie haben wir das Potential von Polysol, einer neu entwickelten Konservierungslösung, zur kalten Lagerung des Dünndarmtransplantates untersucht und mit den aktuellen Standards, der UW-, Celsior- und HTK- Lösung verglichen.

Material und Methoden:

Männliche Wistar Ratten (250-300g) wurden als Spendertiere verwendet. Die isolierten Dünndärme wurde über die Art. mesenterica superior (SMA) und das Lumen mit Konservierungslösung (UW, HTK, Celsior oder Polysol) durchgespült und 18h bei 4°C gelagert. Die funktionale Integrität der Dünndärme in allen vier Gruppen wurde durch eine isolierte Reperfusion mit oxygeniertem Krebs-Henseleit Puffer über die SMA und eine luminale Perfusion des Dünndarms mit Galactose bei 37°C für 30 min überprüft.

Ergebnisse:

Die mit Polysol konservierten Dünndärme wiesen die höchste ATP Konzentration auf und die niedrigste Freisetzung von LDH. Die Menge an Malondialdehyd, ein Indikator für Lipidperoxidation in Geweben, war ebenso am niedrigsten bei der Polysolkonservierung. Der Sauerstoffverbrauch im Gewebe und die Galactoseabsorption waren bei Polysol konservierten Organen signifikant höher, als mit den übrigen Konservierungslösungen. Interessanterweise führte eine UW-Lagerung zu 10-fach höheren Apoptoseraten, im Vergleich zu den übrigen Konservierungslösungen. Darüber hinaus zeigten histologische und elektronenmikroskopische Aufnahmen, dass die mukosale Villi/Mikrovillibildung und

die Zellorganellen, einschließlich der Mitochondrien, signifikant besser mit Polysol erhalten waren, während Organschädigungen mit den übrigen Konservierunglösungen und am meisten mit UW aufgetreten sind. Obwohl Celsior und HTK in einigen Parametern eine bessere Tendenz der Ergebnisse zeigten, waren die Ergebnisse in keinen Fall signifikant im Vergleich zu der UW-Gruppe.

Schlussfolgerung:

Eine kalte Lagerung in Polysol bietet eine signifikant bessere Konservierung und Vitalität des Dünndarms während der Reperfusion im Vergleich zu UW-Lösung. Daher könnte Polysol eine neuartige Alternative zur Dünndarmkonservierung darstellen.

Introduction

In the last decade, small bowel transplantation (SBTx) has emerged as a life-saving option for patients with intestinal failure and life threatening complications of total parenteral nutrition. Although the recent advances in immunosuppressive agents or regimens, such as Tacrolimus or anti-CD25 antibody, have brought a “break though” in organ transplantation, the patient outcome after SBTx still remains inferior to other organ transplantations, such as heart, kidney or liver (Intestinal Transplant Registry 2003).

Of note, the small bowel is the most perfused organ under physiological conditions, receiving up to 25% of all cardiac output, most of which (up to 90%) is consumed in the mucosa and the submucosa, to sustain its large surface area (up to 100 m^2) and its high turn over rate. This physiological feature of the gut leads, in turn, to the extreme vulnerability of the mucosal layer to ischemia during cold storage (CS). Although intestinal mucosa has high regenerative ability, Takeyoshi et al. demonstrated that morphological recovery of the injured ileal mucosa after 24 h CS requires at least one month (Takeyoshi et al. 2001). This prolonged damage from ischemia/reperfusion injury (IRI) surely participates not only in acute graft rejection but also in various postoperative complications, such as bacterial translocation (BT), endotoxin absorption and long-lasting malnutrition of the recipient. Moreover, there have been lines of evidence demonstrating that the incidence of BT was not different between allogenic and isogenic transplantation, in the latter no immunological rejections occurred (Biffi et al. 1995; Zou et al. 2005). These facts clearly suggest that non-immunological factors, such as IRI, seem to play an important role in the development of BT. Taken all these together, how to protect the mucosal integrity during organ preservation still remains of primary interest to further improve the clinical outcome of SBTx (Menger 2006).

Meanwhile, the current clinical standard for small bowel preservation is cold static storage using the University of Wisconsin solution (UW). Despite its overall acceptance as the first choice for solid organs, UW is unable to effectively preserve the small bowel grafts (Kokudo et al. 1994; Leuvenink et al. 2005). Small bowel preservation using UW is rather disappointing since the storage time is restricted only to 6-8 h, thus limiting the area of organ delivery. This limitation, at least in part, interferes to select the most proper recipient of HLA matching, even though more suitable candidates are in out of the transportable area. Moreover, with such a relatively short storage duration, the 1-year graft survival after SBTx is still 65% (Middleton and Jamieson 2005). Other commercially-available preservation solutions, such as Histidine-Tryptophane-Ketoglutarate (HTK) and Celsior have not been used in clinical SBTx so far, mainly due to the lack of relevant evidence for their usefulness (Balaz et al. 2004; Burgmann et al. 1996; Leuvenink et al. 2005). Accordingly, there has clearly been an urgent need for developing a novel refinement in small bowel preservation as well as for a reliable comparison study of standard preservation solutions, for successful SBTx.

Recently, a new preservation solution, Polysol, was developed for hypothermic machine-perfusion preservation. Polysol is a lower viscosity solution than UW, while keeping a high oncotic pressure. Furthermore, Polysol contains free radical scavengers, various kinds of amino acids, vitamins and nutrients, all of which certainly counteract the adverse effects during CS. In fact, we have recently demonstrated, using a rat model of steatotic liver preservation, that Polysol exhibited a superior quality of such “marginal” organ preservation also in CS (Hata et al. 2007). These characteristic features of Polysol have led us to hypothesize that this solution might reduce the high susceptibility of the small bowel grafts to IRI, even in simple CS.

The present study was thus designed to investigate the potential of Polysol on preserving the integrity and the function of small bowel grafts in CS, and to compare its efficacy and feasibility with the current standards, UW, Celsior and HTK.

Materials and Methods:

Animals

All animal experiments were performed in accordance with the federal German law regarding the protection of animals. This study also complied with institution guidelines as well as the criteria in "Guide for the Care and Use of Laboratory Animals" (NIH publication 86-23, revised 1985).

Male Wistar rats weighing between 250 and 300 g were used as donors, and randomly assigned into four groups; UW, Celsior, HTK or Polysol group (n = 7 each). They all received humane care and had an acclimatization period of at least 1 week under specific pathogen free conditions according to the FELASA (Federation of European Laboratory Animal Science Associations) recommendations.

Preservation Solutions

UW (Via Span) was purchased from DuPont Pharma (Bad Homburg, Germany).

The HTK solution (Custodiol), from Dr. Franz Köhler Chemie GmbH (Alsbach-Hähnlein, Germany), Celsior from Genzyme (Neu-Isenburg, Germany), and Polysol from Doorzand Medical Innovations (Amsterdam, The Netherlands) were all provided free of charge for research purpose.

The main components in the respective solutions are summarized in Table 1.

Table 1: Composition of UW, Celsior, HTK and Polysol

Components	UW	Celsior	HTK	Polysol
Colloid (g/L)	HES (250 ku), 50	—	—	PEG (35ku), 20
Na/K ratio (mmol/L)	27/125	100/15	15/10	135/5
Buffers (mmol/L)	H_2PO^{4-} , 25	Histidine, 30	Histidine, 180	Histidine, 6.3 H_2PO^{4-}, 21.74 HEPES, 20
Antioxidants (mmol/L)	Allopurinol, 1 Glutathion, 3	Glutathion, 3	—	Allopurinol, 1.2 Glutathion, 3 Alpha-tocopher ol, 5x10-5 Ascorbic acid, 0.11
Nutrients (mmol/L)	—	—	Glucose, 28	Glucose, 11.1 Adenine, 5 Sodium pyruvate, 0.23
Impermeants (mmol/L)	Lactobionate, 100 Raffinose, 30	Lactobionat e, 80		Raffinose, 3 Trehalose, 5.3 Na^+-Gluconate, 74.99 K^+-Gluconate, 20
Amino acids (mmol/L)	—	Glutamic acid, 20	Tryptophan, 2	various (a), 11
Vitamins (mmol/L)	—	—	—	various (b), 0.17
Ca^{2+} /Mg^{2+} (mmol/L)	—	0.25/13	0.015/4	2/4
others (mmol/L)	Adenosine, 5	Mannitol, 60	Mannitol, 30 Ketoglutarate, 1	Adenosine, 5

pH	7.4	7.3	7.2	7.4
Osmolarity (mOsm/L)	320	320	310	320
viscosity at 5°C (cP)	5.7	1.3	1.8	1.8

Abbreviations: UW, University of Wisconsin solution; HTK, histidine-tryptophan-ketoglutarate solution; HES, hydroxyethyl starch; PEG, polyethylene glycols; cP, centi-Poise.

(a): The following amino acids (mmol/L) are supplemented in Polysol: alanine (1.01), arginine (1.18), asparagines (0.08), aspartic acid (0.23), cystine (0.33), cystine (0.25), glutamic acid (0.34), glutamine (0.002), glycine (0.67), isoleucine (0.38), leucine (0.57), lysine (0.48), methionine (0.30), ornithine (2.00), phenylalanine (0.30), proline (0.78), serine (0.29), threonine (0.34), tryptophan (0.88), tyrosine (0.19), and valine (0.43).

(b): The following vitamins (mmol/L) are supplemented in Polysol: ascorbic acid (0.11), biotin (0.21), Ca-pantothenate (0.004), choline chloride (0.01), inositol (0.07), ergocalciferol (3×10^{-4}), folic acid (0.002), menadione (4×10^{-5}), nicotinamide (0.01), nicotinic acid (0.004), pyridoxal (0.005), riboflavin (0.003), thiamine (0.03), vitamin A (3×10^{-4}), vitamin B12 (1×10^{-4}) and vitamin E (5×10^{-5}).

Retrieval of the Small Bowel

Prior to the surgical procedures, rats were fasted for 24 h while having free access to water, and then anesthetized by intramuscular injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). The abdomen was opened by a midline incision with bilateral subcostal extensions, and the total jejunum and ileum was isolated with the vascular pedicle, as described in detail elsewhere (Minor et al. 1998). The rats were heparinized (1000 U/kg) before the organ retrieval. The superior mesenteric artery

(SMA) was cannulated with a 20-gauge polyethylene catheter and the small bowel was immediately flushed with 10 mL of the respective solutions at 4°C. The portal vein was cannulated with a polyethylene catheter (I.D. 1.5mm), later connected to a silicone tube to allow the collection of the venous effluent upon reperfusion. A short 14-gauge cannula was inserted into the upper jejunal lumen and secured with a circumferential tie. The intestinal lumen was first flushed with 20 mL cold normal saline solution, followed by a rinse with 15 mL of the respective preservation solutions.

Cold Storage

The excised small bowel grafts were stored ischemically for 18 h in a cold bath of the respective solutions (100 mL). The temperature was kept at 4°C constantly by an external cooling circuit (Ministat 125, Peter Huber Kältemaschinenbau GmbH, Germany).

Isolated Reperfusion

The grafts were thereafter reperfused in vitro in a recirculating fashion at a constant flow rate of 8 mL/min for 30 min, according to the previously established techniques (Minor et al. 1998). The grafts were weighed, and then gently reflushed with 5mL of normal saline at 22°C via SMA. They were then transferred into a temperature-controlled (37°C) organ bath, filled with the modified Krebs-Henseleit buffer (KHB). The compositions were as follows: 50 g/L dextran 78, 9.5 g/L KHB, 0.37 g/L calcium chloride, 0.06 mg/L dexamethasone, 0.07 g/L atropine, and 0.21% sodium bicarbonate. The small bowel graft was floated but almost completely immersed in KHB at 37°C, allowing homogeneous perfusion flow and temperature. The care was taken to avoid unnatural bending or distortion of the graft. Carbogen (95% O_2 + 5% CO_2) was used for oxygenation, and perfusate-pO_2 was continuously kept over 500 mmHg during the whole reperfusion period. The intestinal lumen was perfused at the same time with a rate of 0.5 mL/min with saline solution containing

200 mg% of galactose at 37°C.The venous effluent was collected intermittently through the portal vein catheter for biochemical analysis.

Parameters

Adenosine Triphosphate (ATP) concentration

At the end of reperfusion, tissue samples in all groups were snap frozen in liquid nitrogen and preserved below -80°C until later analysis. The samples were first cut into small pieces in liquid nitrogen and weighed (wet-weight), then freeze-dried in a high vacuum system at -40°C for six days (Beta 1-16, Martin Christ Gefriertrocknungs-Anlagen GmbH, Osterode, Germany). After tissue water was evaporated, the samples were weighed again (dry-weight). The tissue concentrations of ATP were determined by standard enzymatic tests, as described in detail elsewhere (Tolba et al. 2001). The results were ultimately corrected with the respective wet-weight per dry-weight ratio of the samples, and expressed in micromoles per gram of dry-weight.

Lactate Dehydrogenase (LDH) Release

Effluent activities of LDH were determined at the end of reperfusion using a commercially-available photometric kit (Boehringer, Mannheim, Germany), as an index for graft tissue damage.

Tissue Lipid Peroxidation

At the end of reperfusion, tissue samples in all groups were snap frozen in liquid nitrogen and preserved below -80°C. Tissue malondialdehyde (MDA) was extracted at 4°C in 10mL/g frozen wet-weight of 0.33 mol/L perchloric acid ($HClO_4^2$), and then neutralized with 2 mol/L potassium hydroxide (KOH). After removing precipitated

$KClO_4$, the MDA concentrations were determined using a fluorometric method, as detailed elsewhere (Mihara and Uchiyama 1978).

Oxygen consumption

The oxygen consumption of the small bowel tissue was determined as a parameter for the remaining metabolic activity of the grafts. Perfusate samples at 30 min reperfusion were taken both from the SMA inflow and from the portal venous effluent, and their oxygen contents were measured immediately by a pH-blood gas analyzer (ABL 5, Acid-Base Laboratory, Radiometer, Copenhagen, Denmark). The oxygen uptake was calculated from the difference between the both samples and expressed as µL O_2 per minute per gram dry-weight, according to the perfusion flow, tissue mass and the solubility coefficient (24 µL of O_2 per milliliter buffer).

Intestinal carbohydrate absorption

Mucosal absorptive function was assessed by calculating the overall uptake of galactose transferred from the intestinal lumen to the portal vein effluent. Effluent concentrations of galactose were measured in the collected perfusate with galactose dehydrogenase using a commercialized test kit (Boehringer, Mannheim). Since galactose is presumed to share a common carrier system with glucose, its uptake form the intestinal lumen may be considered a useful parameter to approximate mucosal carbohydrate absorption.

Apoptosis

Perfusate samples at 15 minutes of reperfusion were also tested for fragmented DNA release using a Cell Death Detection ELISAPLUS Kit (Roche Molecular Biochemicals, Mannheim, Germany), according to the manufacturer’s instruction.

Histology

After reperfusion, specimens were rapidly fixed in 10% buffered formaldehyde and embedded in paraffin. Three-micrometer sections were stained with hematoxylin-eosin for routine histology.

Electron microscopy

At the end of CS, additional tissue samples (n = 3 each) were perfused with glutaraldehyde and paraformaldehyde solution (2% each in phosphate-buffered saline) through SMA with a constant pressure of 70 cmH_2O, then immersed into the same solution for at least 2 days at 4°C. The samples were cut into 0.5 mm slices, post-fixed with OsO_4 (osmic acid fixative) and embedded in Epon 812 (Serva, Heidelberg, Germany). Semi thin sections were then stained according to the procedure by Richardson et al (RICHARDSON et al. 1960). Thin sections were thereafter stained with uranyl acetate and lead citrate, and then examined with a Phillips CM 10 electron microscope (Phillips, Eindhoven, The Netherlands).

Statistics

All results are expressed as mean ± SE from the 7 independent observations, unless otherwise mentioned. Comparisons among the 4 groups were performed by one-way analysis of variance (ANOVA), followed by Tukey-Kramer's post hoc test. The differences were considered statistically significant when *p*-values were less than 0.05.

Results:

Tissue ATP content

The tissue ATP concentration of the graft after CS and oxygenated reperfusion is regarded as one of the most important parameters reflecting the organ viability. We determined the tissue ATP contents at the end of reperfusion in all groups (Fig. 1). The value in Polysol (2.480 ± 0.036 mol/g dry-weight) were highest, and was significantly higher than UW (1.488 ± 0.119 mol/g dry-weight, $p < 0.001$) and in Celsior (1.885 ± 0.143 mol/g dry-weight, $p < 0.05$). The value in HTK (2.031 ± 0.156 mol/g dry-weight) was also significant higher than UW ($p < 0.05$).

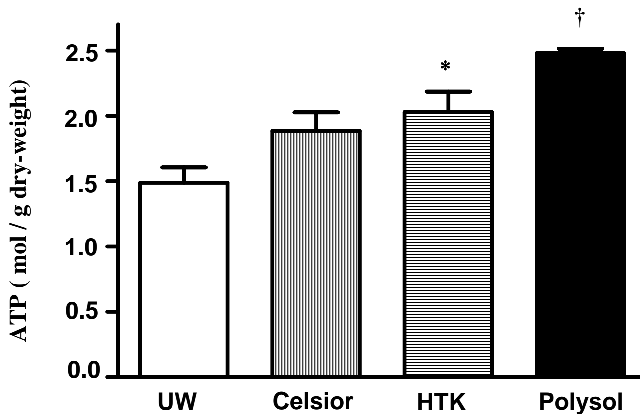


Figure 1: Tissue ATP Content as a parameter for functional recovery of the mitochondria and resultant tissue integrity after 18 h cold storage and 30 min oxygenated reperfusion. Data are expressed as micromoles per gram of tissue dry-weight (mean ± SE, n = 6 each). *: $p < 0.05$ vs. UW, †: $p < 0.001$ vs. UW, $p < 0.05$ vs. Celsior

LDH Release

After 18 h CS and 30 min oxygenated reperfusion, LDH release in UW (69 ± 9.4 U/L) was the highest, followed by HTK (43 ± 7.3 U/L) and Celsior (38 ± 8.4 U/L). The release was the lowest in Polysol (32 ± 6.5 U/L) among the 4 groups. Only the value in Polysol reached the statistically significant level versus UW ($p < 0.05$, Fig. 2).

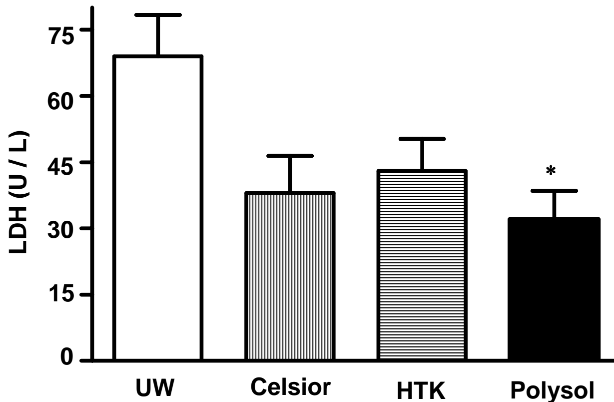


Figure 2: LDH release into the perfusate, as a parameter for general tissue damage after 18 h cold storage and 30 min oxygenated reperfusion (mean ± SE, n = 7 each). *: $p < 0.05$ vs. UW.

Lipid Peroxidation

Tissue MDA content in UW was 9.55 ± 1.360 nmol/mL, which was significantly higher than the other 3 groups ($p < 0.001$ vs. the others). The values in Polysol (2.34 ± 0.731 nmol/mL). HTK (1.47 ± 0.452 nmol/mL) and Celsior (1.33 ± 0.460 nmol/mL) were not significantly different (Fig. 3).

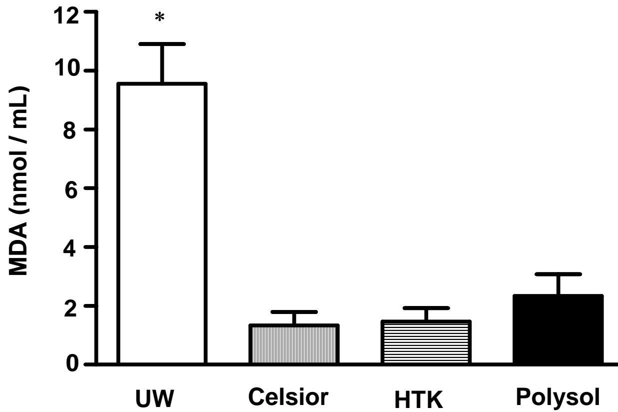


Figure 3: MDA concentrations in the graft tissues, as an index for lipid peroxidation after 18 h cold storage and 30 min oxygenated reperfusion (mean ± SE, n = 6 each). *: $p < 0.001$ vs. Celsior, HTK and Polysol.

Oxygen consumption

The levels of tissue oxygen uptake were not different among the 3 groups (UW: 9.65 ± 0.83, Celsior: 10.63± 0.80, HTK: 9.89 ± 0.46 l/min/g dry-weight). The value in Polysol (13.94 ± 0.51 l/min/g dry-weight) showed the highest among the 4 groups (Fig. 4), reaching the significantly higher level vs. Celsior, HTK ($p < 0.01$) and UW ($p < 0.001$).

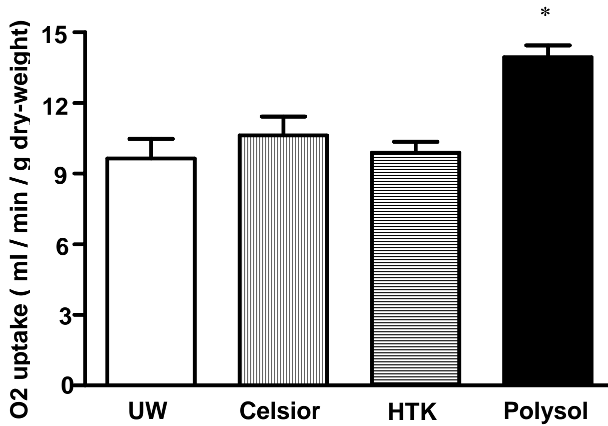


Figure 4: Tissue oxygen consumption at 30 min oxygenated reperfusion, as a parameter for the remaining metabolic activity of the grafts (mean ± SE, n = 7 each). *: $p < 0.01$ vs. HTK and Celsior, $p < 0.001$ vs. UW

Cumulative Galactose absorption

Intestinal carbohydrate absorption, in our model, transportation of Galactose from the intestinal lumen into the vascular system, was measured as viability parameter. The concentration of Galactose in the portal venous effluent was calculated. Our results showed, that during 15min reperfusion the cumulative Galactose absorption in the Polysol (410 ± 22.4 mg %) and the Celsior group (511 ± 53.7 mg %) were higher than in UW (314 ± 13.8 mg %, $p < 0.05$) and HTK group (340 ± 25.5 mg %, $p < 0.05$), the differences are statistically significant.

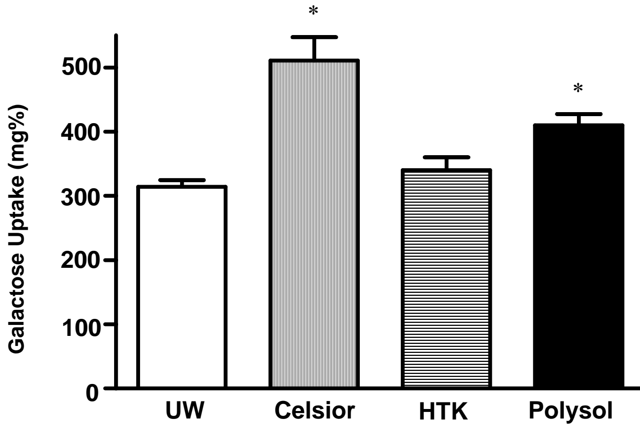


Figure 5: Cumulative Galactose absorption during 30 min oxygenated reperfusion, (mean ± SE, n = 7 each). *: $p < 0.05$ vs. UW and HTK.

Apoptosis

To quantitatively assess the magnitude of apoptosis, we performed a DNA fragmentation ELISA. The highest apoptotic cell death was detected in the UW group (1014 ± 268 AU/mL), which was significantly higher than the other 3 groups ($p < 0.01$ vs. HTK, $p < 0.001$ vs. Celsior and Polysol). The lowest apoptosis was detected in Polysol (81 ± 17 AU/mL), followed by Celsior (123 ± 17 AU/mL) and HTK (172 ± 20 AU/mL). Although statistically not significant to Celsior and HTK, Polysol group showed the lowest values, while 10-fold higher apoptosis was identified in UW (Fig. 5).

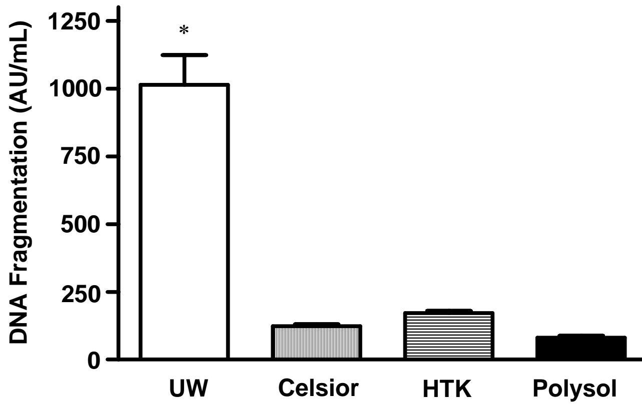
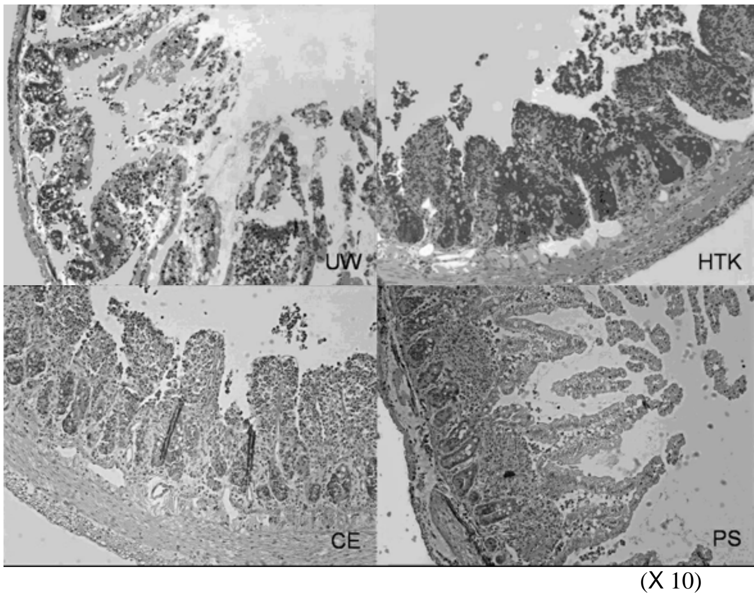


Figure 6: The release of fragmented DNA into the perfusate at 15 min of reperfusion using a Cell Death Detection ELISA kit (mean ± SE, n = 6 each). *: $p < 0.01$ vs. HTK, $p < 0.001$ vs. Celsior and Polysol.

Histology

To estimate the structural integrity of SB after 30min reperfusion, the specimens were fixed with formalin and stained with hematoxylin and eosin.



(X 10)

Figure 7: Histological evaluations of the cold-stored organs. At the end of the reperfusion, the mucosa in the UW group was almost entirely denudated, and there were separations between the submucosa and the muscular layer. The structural integrity in HTK and Celsior group were better than in the UW group, but showed significant more damage compared to the Polysol group. The best-preserved structural integrity was seen in the Polysol group.

Electron microscopy

In order to evaluate the graft tissue damage at ultrastructural level, especially focusing on the microvilli formation as well as on the cell organelles in enterocytes, we conducted electron microscopy after 18 h CS, as illustrated in Figure 8.

The microvilli formation in UW were severely damaged, showing sparse and lowered-height of microvilli (Figure 8-A). In Celsior, although the microvilli density

was relatively better preserved than in UW, the height of microvilli was apparently lowered to the same level in UW (Figure 8-B). In HTK, the height of microvilli was better preserved than in UW and in Celsior, however, the breakdown of their apical region was diffusely noted, displaying ragged and jagged formation (Figure 8-C). In contrast to the other 3 groups, such deleterious alterations were rarely visible in Polysol, indicating homogeneously preserved microvilli epithelia of the luminal surface (Figure 8-D).

With regard to the damage in cell organelles, the mitochondria in UW displayed mild swelling and less electron density of their matrices, in which the cristae were hardly visible (Figure 8-E). In Celsior and HTK, such detrimental alterations in mitochondria were also identified, in spite of relatively better-preserved electron density of the ultrastructures (Figure 8-F, -G). Unlike the other groups, the mitochondria in Polysol kept their oval shape with well-maintained cristae formation (Figure 8-H). These findings are thus in good agreement with the results of tissue oxygen consumption and the resulting ATP contents, at the point of mitochondrial damage and function.

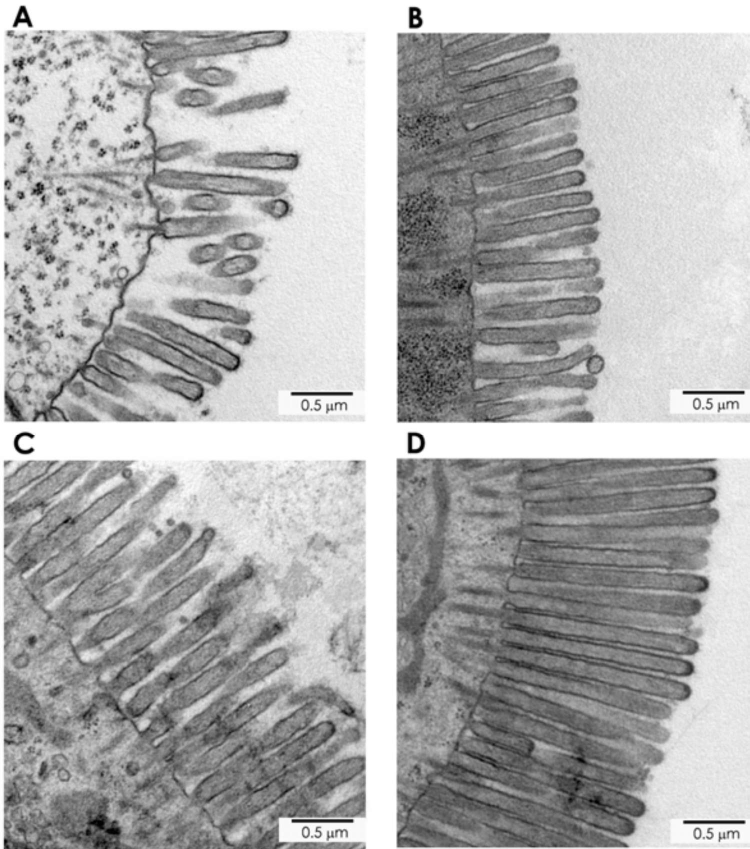


Figure 8-A to -D: Ultrastructural Analysis using electron Microscopy, Microvilli condition after 18 h CS. Scale bar: 0.5 µm

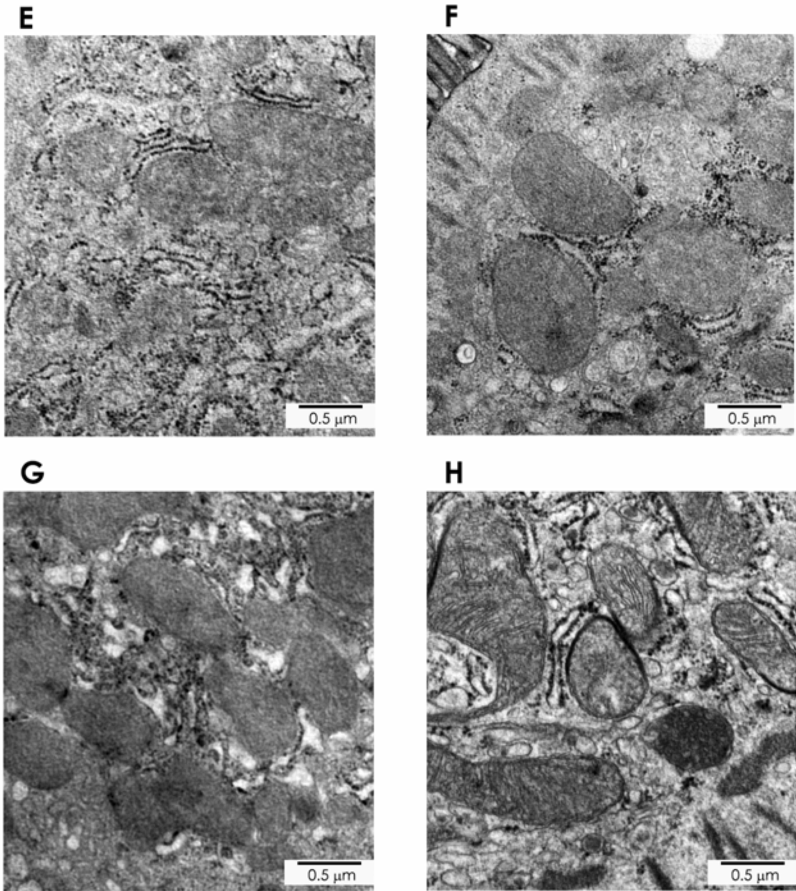


Figure 8-E to -H: Ultrastructural Analysis using electron Microscopy, Mitochondria in Enterocytes after 18 h CS. Scale bar: 0.5 μm

Discussion

Unlike other organs, small bowel transplantation (SBTx) has several characteristic burdens, such as rich lymphoid tissues and large mucosal surface expressing class-2 major histocompatibility antigens. Indeed, the recent achievements in immunosuppressants, including the development of Tacrolimus, had brought a new era also in the management and outcome of clinical SBTx (bu-Elmagd et al. 2001). Thus, uncontrollable immunological rejection, which had long been the main cause of patient death, was superseded by septic complications as the main cause of mortality (Intestinal Transplant Registry 2003; Middleton and Jamieson 2005). These events then emphasize another aspect of high hurdles in SBTx: How to preserve the mucosal integrity and the barrier functions during/after organ preservation.

In the present study, we demonstrated that cold storage (CS) of small bowel grafts using Polysol resulted in significantly better quality of graft preservation than the current clinical standard, UW, in all the parameters we tested. Surprisingly, UW exhibited the worst trend of results in most parameters, even compared with other currently available solutions, HTK and Celsior. In particular, morphological integrity of the mucosa, both microvilli formation and cell organelles, such as mitochondria, were significantly better preserved by Polysol, whereas detrimental alterations were apparent in the UW-stored grafts. Although the present study did not elucidate the exact mechanisms of the benefits by Polysol, as well as of the worst results by UW, one possible explanation to this difference is, at least in part, in numerous amino-acid supplementations in Polysol.

Polysol contains up to 21 kinds of amino acids, including glutamine, glutamic acid, asparagines, aspartic acid, glycine, and so on (Table 1). During CS, these numerous amino acids play protective roles in a variety of cellular house keeping processes by catering both for metabolic (energy production) and for synthetic (synthesis of critical

molecules) aspects of intestinal metabolism (Windmueller and Spaeth 1980; Wu 1998). Among them, glutamine, glutamate, and aspartate are crucial for enterocytes as major energy sources (Alpers 2000). The intestinal glutamine catabolism is a multistep process, resulting in ATP production via the TCA cycle or via the provision of key elements to the nitrogen-carbon backbone of purines. Since the glucose utilization is limited during CS, glutamine supplementation is thought to be effective to maintain cellular ATP level, thus alleviating the tissue damage (Olson et al. 2002). Furthermore, because small intestinal mucosa becomes atrophic when the gut is deprived of glutamine, its supplementation to preservation solutions surely counteracts the mucosal atrophy during/after cold storage, as manifested by electron microscopy. Glutamic acid also augments energy production in anaerobic conditions (Minor et al. 1998). Moreover, glycine is expected to work against oxygen species and stabilize the tertiary protein structure of cell membranes upon reperfusion (Zhong et al. 2003). Also, glycine can restore cellular ATP levels (Deters et al. 1997). As already reported, amino-acid based solutions could provide a better energy storage in the small bowel preservation (Salehi et al. 2003; Tsujimura et al. 2004).

Another characteristic difference is noticed in the anti-edematous ingredients (Table 1). Different from the others, Polysol contains polyethylene glycol (PEG) that enables both the required oncotic pressure and the relatively low viscosity (1.8 cP). PEG stabilizes lipid membranes and contributes to less membrane permeability (Daniel and Wakerley 1976; Robinson 1971), thus preventing osmotic cell swelling and vascular endothelial damage (Hauet et al. 2001a). Moreover, PEG also act as a OFR scavenger, markedly reducing lipid peroxidation upon preservation/reperfusion process (Hauet et al. 2001b; Mack et al. 1991). In UW, the colloid used is hydroxyethyl starch (HES), which (5% in UW) causes over 3-fold higher viscosity (5.7 cP), thereby triggering vasoconstriction and endothelial damage, in cooperation with the high potassium concentration (Mankad et al. 1992; Pearl et al. 1994). Moreover, HES also causes hyperaggregation of erythrocytes, which may result in incomplete, heterogeneous blood-washout of the graft tissue. On the other hand, HTK and Celsior are crystalloid

solutions with lower viscosity and lower potassium concentration (Table 1). It was already proven that HTK and Celsior were superior than UW as an initial flushing solution, not only for small bowel but for liver and heart (Muller et al. 1994; Puhl et al. 2006), mainly due to the lower viscosity of 1.3 – 1.8 cP. The viscosity of Polysol is also 1.8 cP, and was proven to have the same efficacy to HTK as a flushing solution (Bessems et al. 2005).

Among the parameters we investigated, one of the most remarkable differences is notable in the magnitude of apoptosis. Surprisingly, the apoptosis in the UW-stored grafts was almost 10-fold higher than that in Polysol. Apoptosis affects the permeability of the intestinal mucosa, impairs its barrier function after transplantation, thus leading to BT and endotoxin absorption. As previously demonstrated, apoptosis is a predominant form of cell death in intestinal IRI (Ikeda et al. 1998), and is an important predictive factor of primary allograft dysfunction in clinical SBTx (Oberbauer et al. 1999). Considering the mechanisms underlying the difference in apoptosis, we focused on the antioxidative properties in each solution. As presented in Figure 4, Lipid peroxidation, caused by oxygen free radicals (OFR), in UW group was almost 5-fold higher than the other groups. Polysol has optimized OFR scavenging potential, achieved by supplementation of not only glutathione and allopurinol but also ascorbic acid (vitamin C), selenium, glycine and alpha-tocopherol (vitamin E), all of which were proven to be effective as antioxidants upon organ preservation (Albuquerque et al. 2002; Mutlu-Turkoglu et al. 2000; Yin et al. 2002). Celsior and HTK, include relatively high dose of histidine (Table 1), which neutralizes reactive oxygen species and preserves high-energy phosphates (Cai et al. 1995). Thus the enhanced antioxidative property of these 3 solutions attenuated OFR-mediated cellular damage upon reperfusion, thereby reducing apoptotic cell death.

Although further investigations in a transplant model are of course required to confirm the findings in this study, this ex vivo experimental setting has several advantages to assess the quality of organ preservation: First, in contrast to a real transplant model,

the results from this isolated setting are not influenced from immunological alloreactions, facilitating the precise evaluation for the organ preservation. Taken into account that intestinal mucosa is the main target from the host immune system, because of its high expression of class-2 major histocompatibility antigens, the isolated setting used in this study is thought to be feasible to assess the quality of organ preservation itself, without any interference from immunological reactions. Simpler procedures (just organ retrieval, without implantation), resultant well-reproducible results and less victims of animals (no recipient is needed), are also the advantages of this method. In summary, this is the first report demonstrating a comparison study of the currently available preservation solutions, UW, HTK, Celsior and Polysol, in the efficacy for small bowel preservation. Polysol, a newly developed preservation solution, exhibited the superior quality of small bowel preservation than the current clinical standard, UW, supported by higher energy potential, less lipid peroxidation, higher metabolic activity and better morphological integrity after 18 h cold storage. HTK and Celsior also showed better potential in some parameters, however, the both could not reach the over all superiority than UW. Hence in conclusion, Polysol may be a novel suitable alternative for small bowel preservation.

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