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REVIEW

Small bowel preservation for intestinal transplantation: a review

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Keywords
intestinal preservation, intestinal transplantation, luminal preservation, organ viability, preservation injury, preservation solution and composition.

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Summary

Intestinal transplantation has become the therapy of choice for patients with intestinal failure and life-threatening complications from total parenteral nutrition. Results, however, remain inferior as compared with other transplant types with the quality of the organ graft as the most important factor of outcome after transplantation. The intestine is extremely sensitive to ischemia. Unfortunately, a relatively long ischemic preservation period is inevitable. The current standard in organ preservation [cold storage (CS) with University of Wisconsin solution] was developed for kidney/liver preservation and is suboptimal for the intestinal graft despite good results for other organs. This review aimed at appraising the results from the use of previously applied and recently developed preservation solutions and techniques to identify key areas for improvement. As the studies available do not reveal the most effective method for intestinal preservation, an optimal strategy will result from a synergistic effect of different vital elements identified from a review of published material from the literature. A key factor is the composition of the solution using a low-viscosity solution to facilitate washout of blood, including amino acids to improve viability, impermeants and colloids to prevent edema, and buffer for pH-homeostasis. Optimizing conditions include a vascular flush before CS and luminal preservation. The most effective composition of the luminal solution and a practical, clinically applicable optimal technique are yet to reach finality. Short-duration oxygenated arterial and/or luminal perfusion have to be considered. Thus, a tailor-made approach to luminal preservation solution and technique need further investigation in transplant models and the human setting to develop the ultimate technique meeting the physiologic demands of the intestinal graft during preservation.

Introduction

Intestinal transplantation (ITx) has become an established treatment for intestinal failure (IF) when parenteral nutrition (PN) fails [1]. ITx is a challenging procedure. Long-term outcome remains inferior as compared with other solid organ transplants, despite significant improvement over the past 20 years [2,3]. Postoperative infectious complications and rejection are the main limiting factors. Results need further improvement to replace PN as primary treatment for IF as long-term PN is associated with life-threatening complications and a poor quality of life.

Graft viability prior to implantation is a key factor in the outcome after organ transplantation [4,5]. Along with brain death in the donor, surgical manipulation, and ischemia-reperfusion injury (IRI), preservation damage is one of many essential factors that affect the quality of the intestinal graft and its barrier function [6–10]. The

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compromised barrier function induces inflammatory upregulation and bacterial translocation (BT), predisposing the recipient to rejection and infectious complications (Fig. 1).

The intestinal mucosa is extremely vulnerable to injury resulting from hypoperfusion [11,12]. Unfortunately, ischemia is inevitable during the preservation process that is in-built, in the face of the gap between availability of donor organs on the one hand and prospective recipients on the waiting list on the other. In Fig. 2, the key principles of intestinal preservation are illustrated.

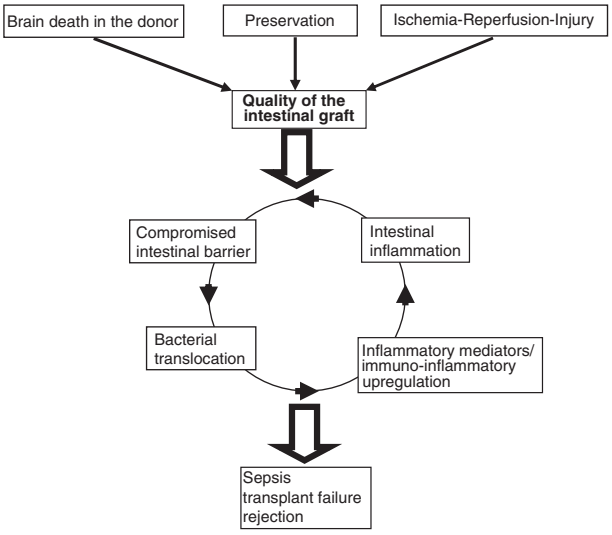

Figure 1 Hypothetic relation between graft damage and outcome after intestinal transplantation.

The lack of an adequate strategy to preserve the intestinal graft allows only a short (6–10 h) preservation span and results in variable degrees of tissue injury [13] limiting the clinical success of ITx. Better intestinal preservation (IP) is a first step to improve the results of ITx.

The intestine has the complex and dual tasks of digesting and absorbing nutrients while maintaining a selective barrier against the external environment. The intestinal mucosa is composed of surface-increasing, fingerlike (villous), absorptive columnar epithelium covering the lamina propria, which hosts blood vessels and lymphatics. In the crypts, at the base of the villi, the intestinal stem cells reside, which are pivotal for regeneration and repair. The absorptive columnar enterocyte is the main epithelial cell: characterized by its apical microvilli which contain transport proteins and digestive enzymes. The effectiveness of the intestinal transcellular barrier is primarily based on the functioning of selectively permeable epithelial enterocytes. Passive paracellular passage *between* cells, however, would nullify this transcellular barrier in the absence of the *inter*cellular sealing junctions. Especially the tight junction proteins (TJ) are critical for a proper barrier function. Unfortunately, these pivotal epithelial enterocytes and TJ are very sensitive to hypoperfusion [8,14]. Effective IP should therefore protect enterocytes, epithelial crypts and conserve TJ.

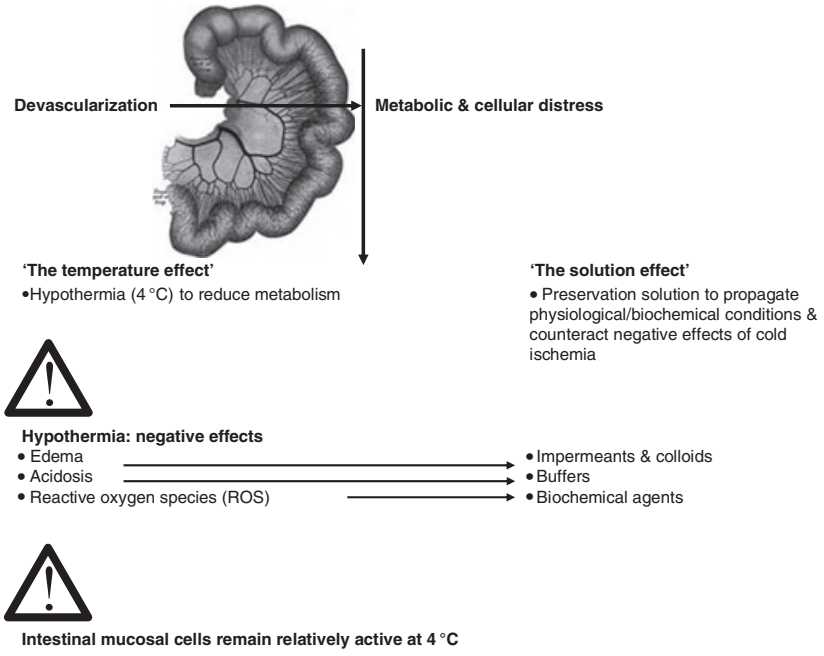

Figure 2 Principles of intestinal preservation.

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In the 1980s, the University of Wisconsin (UW) solution was introduced by Belzer and Southard. UW is an intracellular type of preservation solution based on the trisaccharide raffinose and the anion lactobionate as osmotic impermeants, hydroxyethyl starch (HES) as colloid, a phosphate buffer, glutathione and allopurinol as reactive oxygen species (ROS) scavengers and the ATP precursor adenosine (Table 1). Vascular wash-out and CS with UW is currently considered the gold standard for preservation of abdominal organs [15,16]. This regime effectively protects kidney, pancreas and liver but less sufficiently protects intestinal integrity and function [17–19]. Adequate preservation of the intestine should therefore be performed in two ways: using intravascular and intraluminal preservation. Optimally modified composition of the preservation solution and technique appropriate for IP have yet to be identified. The aim of this review was to clarify demands of the intestinal graft during preservation to achieve a tailor-made intestinal-specific preservation policy. To identify key factors for improvement, this article appraised the results of studies undertaken for developing alternative strategies reported in the literature. Table 2 outlines the characteristics of the studies reviewed.

Preservation solutions: components and characteristics

Electrolyte composition

Originally, an intracellular electrolyte ratio with high potassium and low sodium was thought to prevent cellular edema because of alternate electrolyte-transport during hypothermia. Therefore, the original UW solution is an example of such an intracellular like solution. Over time and because of more insight, it was demonstrated that extracellular like solutions containing high sodium and low potassium were more effective in maintaining the Donnan equilibrium of the cell membrane, without potassium-induced vasospasm.

Celsior is a simple, UW-like *extracellular type* preservation solution differing from UW by its monosaccharide impermeant mannitol, the buffer histidine and absence of a colloid. Minor *et al.* [20] described that Celsior provided better postischemic (18 h) intestinal graft recovery than UW in the rat, with less vascular resistance upon reperfusion, lower lactate production and better carbohydrate absorption. The differences in reperfusion pressure have possibly caused the encountered metabolic and functional differences although no histologic differences were seen. Prolonged vasoconstriction and increased pressure upon reperfusion in reaction to the high-potassium concentration of UW was proposed to have a deleterious impact on vascular endothelium [21,22]. deRoover *et al.* [23] compared the histology of *human* tissue after either UW or Celsior vascular flush followed by 24 h CS. However, in line with the above results, no histologic differences could be demonstrated.

Histidine tryptophan ketoglutarate (HTK) is a more distinct, extracellular type, low-viscosity solution. HTK is based on the impermeant mannitol, the buffer histidine and two amino acids (AA): tryptophan which is membrane-stabilizing and anti-oxidative, and ketoglutarate, a substrate for anaerobic metabolism (Table 1). HTK is postulated to have clinical advantages over UW (e.g. easier diffusion and faster cooling during wash-out). Clinical results regarding the superiority of HTK over UW, however, are controversial [16]. Mangus *et al.* [24] compared clinical results of ITx after use of either HTK or UW for preservation. No differences were seen in initial graft function, endoscopic appearance and rejection episodes, although a better blood wash-out was seen with HTK during intestinal procurement. This study has important limitations: short follow-up (maximum 90 days) and lack of randomization.

The new, extracellular type Polysol is a complex experimental solution, which has a high oncotic pressure and a three-time lower viscosity than UW. Polysol contains the colloid polyethylene glycol (PEG), the impermeants raffinose and gluconate, a phosphate buffer, the buffer histidine and a sulfonic buffer 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), a variety of ROS scavengers, 21 different AA and 16 different vitamins (Table 1). Wei *et al.* [25] compared rat intestinal graft quality after CS (18 h) with UW, HTK, Celsior and Polysol followed by 30 min of *in vitro* reperfusion. Polysol exhibited higher ATP levels, lower lactate dehydrogenase (LDH) production, higher tissue-oxygen consumption and a better preserved microstructure (e.g. mitochondria) versus UW. However, the much simpler HTK and Celsior also showed similar benefits. Malondialdehyde, reflecting peroxidation, and apoptotic cell death was higher with UW than other solutions. Also, ATP levels were higher for HTK versus UW. The extracellular character of Celsior, HTK and Polysol could be related to the superior results. Because of the model of *ex-vivo* reperfusion in the rat and the short reperfusion period, the clinical impact of these findings remains limited.

Muller *et al.* [26] evaluated the two commercially available solutions UW and Euro Collins, and in addition a variety of fundamental solutions with different electrolyte compositions: phosphate buffered sucrose (PBS, containing sodium), extracellular fluid (ECF, high potassium-low sodium), lactobionate fructose (LF, high potassium-low sodium), and saline (high sodium) were tested for their effectiveness in rat IP (12 h). This study appraised

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Table 1. Composition of preservation solutions.

Components mmol/l (or indicated otherwise)	EC [14]	UW [14]	CLS [19]	HTK [24]	Polysol [24]	AA solution example [61]
Colloids/impermeants						
Glucose	195				11.1	20
HES (g/l)		50				
K^+-Gluconate					20	
Lactobionate		100	80			100
Mannitol (MW 182)			60	30		
Na^+-Gluconate					74.99	
PEG (35) (g/l)					20	
Raffinose		30			3	
Trehalose					5.3	
Buffers						
BES						90
H_2PO_4					21.7	
HEPES					20	
Histidine			30	198	6.3	5
KH_2PO_4	43	25				
K_2HPO_4	15					
$NaHCO_3$	10					
Antioxidants						
Allopurinol		1			1.2	
Alpha-tocopherol					5×10^{-5}	
Ascorbic acid					0.11	
Glutathion		3	3		3	
Sodium pyruvate					0.23	
Tryptophan				2		
Additives						
Adenine					5	
Adenosine		5			5	5
Hydroxy-butyrate						3
Ketoglutarate				1		
Ornithine					2	5
Amino acids						
Alanine					1.01	
Arginine					1.18	10
Asparagine					0.08	10
Aspartate					0.23	20
Cysteine					0.58	5
Glutamate						20
Glutamic acid			20		0.34	
Glutamine					0.002	35
Glycine					0.67	10
Isoleucine					0.38	5
Leucine					0.57	5
Lysine					0.48	10
Methionine					0.3	5
Phenylalanine					0.3	
Proline					0.78	5
Serine					0.29	10
Threonine					0.34	10
Valine					0.88	10
Tryptophan					0.43	1
Tyrosine					0.19	1
Vitamins						
Ascorbic acid					0.11	
Biotin					0.21	

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Table 1. continued

Components mmol/l (or indicated otherwise)	EC [14]	UW [14]	CLS [19]	HTK [24]	Polysol [24]	AA solution example [61]
Ca-pantothenate					0.004	
Choline-Chloride					0.01	
Ergocalciferol					3×10^{-4}	
Folic acid					0.002	
Inositol					0.07	
Menadione					4×10^{-5}	
Niacinamide					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}	
Vitamin E					5×10^{-5}	
Electrolytes						
Magnesium-sulfate		5				
Measured electrolytes						
Calcium			0.25	0.02	2	
Chloride	15	20		32		
Magnesium			13	4	4	
Potassium	115	120	15	10	5	
Sodium	10	25	100	15	135	
Properties (if given)						
pH	7.0	7.4	7.3	7.2	7.4	7.4
Osmolarity (mOsm/l)	355	330	320	310	320	
Viscosity (cP)		5.7	1.3	1.8	1.8	

AA, amino acid; BES, *N*,*N*-Bis(2-hydroxyethyl)-2-aminoethanesulfonic-acid; CLS, Celsior solution; EC, EuroCollins solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HTK, histidine tryptophan ketoglutarate; kDa, Kilo-dalton; MW, molecular weight; PEG, polyethylene glycol; UW, University of Wisconsin solution.

histology and glutaminase activity, which reflects enterocyte integrity, after 20 min reperfusion and 7-day graft survival. No difference in graft survival was observed suggesting that electrolyte composition is probably of minor importance.

Kokudo *et al.* [17] aimed to identify the optimal IP solution composition by evaluating rat survival and graft electrophysiologic functional performance (using an ussing chamber) during18-h preservation. UW, Euro Collins and Lactated Ringer's solution (RL, low potassium–low sodium) were compared. In all solutions, enterocyte function rapidly deteriorated. Crypt cell function was better preserved with RL and EC than with UW. After 12 h of CS, survival was lower in the UW group. After 18 h, however, no differences in survival were seen among the preservation groups.

Summarizing, extracellular like, high-sodium, low-viscosity solutions seem beneficial for wash-out and reperfusion characteristics, but the exact role of sodium-potassium ratio and separate electrolytes in maintenance of intestinal graft viability is unclear.

Impermeant and colloid support

One of the unwanted negative effects of hypothermia is a passive sodium influx into the cytosol followed by water, resulting in tissue edema. To counteract this phenomenon, impermeants and colloids are determined to be the key factors in hypothermic preservation.

University of Wisconsin Solution includes the large trisaccharide impermeant raffinose and the anionic impermeant lactobionate, which is also present in Celsior in combination with the monosaccharide mannitol. Dialysed HES is the colloid component in UW. Its efficacy has been debated because it increases viscosity and could cause red blood cell (RBC) aggregation -both negatively affecting wash-out and reperfusion [27–33]. Alternatively, polysaccharide dextrans and PEG have been applied as colloid agents. PEG was assumed to result is less RBC aggregation and lower viscosity, seemed to stabilize lipid membranes [29] minimizing permeability [28,32], and furthermore acts as a free radical scavenger [29,34]. PEG was proved to be effective for IP [35–38]. Nevertheless, none of the

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Table 2. Characteristics of reviewed studies.

Reference (Author, journal, year)	Purpose/aim Materials and methods Study design: species and total sample size	Materials and methods Study design: Experimental groups	Materials and methods Surgical/experimental procedure	Materials and methods Outcome parameters and assessment time points	Results ($P < 0.05$)	Conclusion	Comments (Strength/ weakness/remarks)
Animal studies							
Rodriguez, J Invest Surg, 1994	Evaluate determinants of failure after 24 h IP and ITx and the effects of different PS IP and Tx study Syngeneic ITX model Spraque-Dawley rat, *N* = 107	PS groups: Control, saline flush No vascular flush, 6 h CS No vascular flush, 24 h CS Vascular flush + CS: EC Vascular flush + CS: UW Vascular flush + CS: ADAAV	Entire small intestine Luminal flush 30–40 ml neomycin + saline +/– *ex-vivo* vascular flush 8 ml RPS, CS 24 h 4–7 °C NB: ITx with bowel ostomy to abdominal wall, POD 10 anastomosis after native bowel excision	Parameters: Lactic acid, SGPT, SGOT, LDH, histology, luminal glucose concentration, survival, clinical evaluation, determinants of failure Time points: 1 h after ITx Survival early/late =3/>3 days	3-day survival 80% control vs. 66.6% ADAAV, 53.5% UW, 46.6% EC, ADAAV = n.s. difference control No survival if no vascular flush Biochemical/histology no difference between PS No >14 day survival for any of PS groups.	No PS can preserve rat intestine under hypothermia for 24 h No survival >14 days in any of PS groups. Factors of failure: IP injury was expressed as hemorrhagic necrosis and sepsis after 24 h of IP	Strength: Studies (causes of) death and graft failure in relation to difference PS. Remark: Best luminal glucose uptake in UW, however, these were not the grafts with best survival.
Muller, Transplantation, 1994	Delineate most optimal PC and PS for IP IP and Tx study Syngeneic heterotopic ITx model Lewis rat, *N* = 66	1st part PC groups: (i) Rewarming before reperfusion (ii) 2nd vascular flush after CS (for all PC CS with ECF) 2nd part PS groups: (in proofed optimal PC) 7 PS groups: UW/ EC/HTK/PBS/ECF/LF/ LF2/saline	Entire small intestine retrieved Lumen rinse 2–3 ml RPS Vascular flush with 1.5 ml RPS + neomycin CS in RPS + neomycin 12 h 4 °C	Parameters: Survival (POD7), histology, glutaminase activity (reflects enterocyte integrity) Time points: 20 min after reperfusion and at POD7.	PC Outcome optimal when vascular flush after CS is omitted and topical rewarming with 37 °C saline i.p. performed: 4/6 survival (67%) for IP PS No difference in survival Histology 20 min reperfusion minor difference b) glutaminase activity >20 min reperfusion for PBS	Different PC have an impact on graft survival and PBS might be preferable to any of the other PS tested	Surplus value: 2-part study, compares PC and PS

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Table 2. continued

Reference (Author, journal, year)	Purpose/aim Materials and methods Study design: species and total sample size	Materials and methods Study design: Experimental groups	Materials and methods Surgical/experimental procedure	Materials and methods Outcome parameters and assessment time points	Results ($P < 0.05$)	Conclusion	Comments (Strength/ weakness/remarks)
Kokudo, Transpl Proc, 1994	Determine optimal PS for ITx IP and Tx study Syngeneic orthotopic ITx model Lewis rat, *N* = unknown	PS groups: Vascular flush and CS UW/EC/RL NB. Rats fasted for 18 h and pretreated with oral neomycin	Entire small intestine Vascular flush 20 ml RPS + luminal flush 40 ml cold NaCl 0.45% + 2.5% glucose + neomycin) CS in RPS for 0, 6, 12, 18 h, 4 °C	Parameters: Survival, electro-physiological enterocyte and crypt cell function (ussing chamber), histology (Park Score) Time points: After CS, 1 h after reperfusion, survival	Survival decreased with CS time At 0, 6, 18 h no difference in survival After CS 12 h CS, less survival for UW than other PS Cell function better RL and C than UW (6/12 h CS)	For rat IP, UW not superior to EC or RL 12 h CS with UW: decreased survival Crypt cell function better with RL/EC than UW	Information lack: Survival duration and numbers of animals
Ito, Transpl Proc, 1995	Effect of luminal GLN to RL on IP graft injury in ITx IP study Mongrel dog, *N* = unknown	PS groups: Vascular/luminal perfused preservation: (i) RL + GLN/RL + GLN (ii) RL + GLN/RL (iii) RL/RL	Jejunum 10 cm segments luminal flush saline + AB 24 h perfusion at 4 °C according to PS groups (vascular/ luminal)	Parameters: Amino acids in perfusate, GLN metabolism Tissue morphology, viability of enterocytes, enterocyte protein synthesis Time points: After 24 h perfused preservation	Villus structure best in group 1: luminal GLN favors epithelial cells at tip of villi, vascular GLN favors crypt cells GLN favors number of epithelial cells, viability and protein metabolism	Optimal PS for the intestine has not been established Luminal + vascular perfused IP with RL + GLN favors mucosal intestinal cells and is useful for IP	Weakness: GLN supplementation concentration not mentioned.

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Mueller, Transpl Proc, 1996	Identify most optimal PC and the role of PS IP and Tx study Syngeneic heterotopic ITx model Lewis rat, $N = 66$	1st part PC groups: (i) Rewarming before reperfusion (ii) Vascular/luminal flush after CS with saline (iii) Temperature 8 °C/4 °C (iv) pH 7.4/6.8 (for all PC groups. CS with ECF) 2nd part PS groups: UW/EC/PBS/HTK/ECF (all groups rewarming, no 2nd flush)	Entire small intestine retrieved Lumen rinse 2–3 ml RPS + neomycin Vascular flush 1 ml RPS. CS in RPS + neomycin 12 h 4 °C	Parameters: Survival, histology, glutaminase activity, Park score Time points: 20 min after reperfusion and at POD7	PC Survival better (4/6) with PC: 4 °C, pH 6.8, no extra vascular/luminal flush after CS, topic rewarming before reperfusion vs. 1/6 no rewarm, 2/6 vascular flush, 0/6 vascular + luminal flush PS Survival UW, EC, ECF (4/6), HTK (3/6), PBS (5/6) Glutaminase activity after 20 min reperfusion better for PBS	Survival depends on PC: vascular washout, vascular + luminal flush after CS decrease survival Topic rewarming with 37 °C saline i.p. beneficial pH 6.8 better than 7.4 Best PC: no flush after CS, pH 6.8, topic rewarming at reperfusion No difference results for difference PS	Flush after CS is detrimental, possibly by mechanical damage to microvasculature Remark: Histologic damage severe in most groups but recovered at POD7
Kokudu, Transpl Proc, 1996	Study the effect of luminal flush on mucosal injury during/after cold ischemia IP + *in vitro* reperfusion study Lewis rat, $N = 18$	PS groups: Vascular flush RL Control: no CS Luminal flush RL No luminal flush CS with RL	Vascular flush RL, +/– luminal flush with RL according to exp. group. CS RL 4 °C 12 h, re-oxygenation in Krebs medium	Parameters: Electrophysiologic function (ussing chamber): PD/R, morphology by EM, apoptosis Time points: after CS and after re-oxygenation	Function (PD) preserved by luminal flush, R not Luminal flush causes more apoptotic changes	Luminal flush decreases mucosal injury but does not prevent apoptosis, More protection is needed for a longer IP span	Remark: Decreased barrier function at 12 h IP as a result of mucosal destruction

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Table 2. continued

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Sasaki, J Surg Research, 1997	To prove that UW + GLN improves intestinal quality after CS IP study Lewis rat, $N = 74$	PS groups: Vascular, luminal flush and CS: Saline UW UW + 1/2/4% GLN UW + 1/2/4% NEAA	Jejunum segment Vascular flush 5 ml RPS, luminal flush 20 ml RPS according to PS groups CS 18 h in RPS 4 °C	Parameters: Glucose transport, mucosal protein, maltase, AF, villous height, histology Time points: After 18 h preservation	Glucose transport better in UW + 2/4% GLN versus UW Histology and mucosal enzymes better in UW + 2/4% GLN (4% not better than 2%) vs UW 4% NEAA increased glucose transport, villous height and mucosal protein versus UW	Addition of 2/4% GLN to UW decreases mucosal damage in rat intestine 2% GLN is optimal for IP	Surplus: Addresses effects of difference concentration of GLN Remark: 4% NEAA superior to UW: osmolarity effect?
Minor, Transpl Int, 1998	Evaluate potential of CLS to protect the intestine during ischemic CS IP and *in vitro* isolated (re)perfusion study Wistar rat, $N = 18$	PS groups: Vascular flush, luminal flush and CS UW/CLS NB. Control samples from vital intestines	Segments 15 cm jejunum. Vascular flush RPS + luminal flush 10–15 ml of RPS CS in RPS 18 h 4 °C Reperfusion *in vitro* (after vascular flush 5 ml NaCl 37 °C) in NaCl organ bath, nonrecirculating vascular flow (5 ml/min) with Krebs + 5% dextran 78 + 95% O_2 + 5% CO_2, luminal flow saline + galactose (0.5 ml/min)	Parameters: Vascular resistance at reperfusion Metabolic and functional integrity (edema, energy, glucose, lactate, creatine phosphate (CP), histology, oxidative tissue injury (ROS), effluent LDH and lipid peroxides (LPO), intestinal galactose absorption, transcapillary fluid loss Time points: After CS, during reperfusion	After CS No difference CLS/UW for edema/energy/CP after CS Lactate less in CLS Histology no difference Reperfusion Vascular resistance and LDH release in perfusate less for CLS and better galactose absorption for CLS	CLS may be a suitable alternative for IP: within limits of *in vitro* pilot study, CLS better post ischemic recovery of intestine than UW (vascular perfusion characteristics, enzyme release, galactose absorption)	Remarks: Tissue edema measured: both PS equally prevent increase in tissue water content Less glucose content for CLS

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Olson, Am J Transpl, 2001	Study the requirements for impermeant (osmotic/oncotic) support in IP IP study Spraque-Dawley rat, *N* = 16	PS groups: Vascular flush and CS NaCl NaCl + 5% Dextran NaCl + 100 mM LB + 5% Dextran UW NB. Rats fasted	*In vivo* vascular flush with RPS Entire intestine retrieved CS 0, 1, 2, 4, 10 h with 30 ml. RPS 4 °C	Parameters: Energetics (ATP/TA), radio-active Mannitol permeability., histology Time points: At t = 0, 1, 2, 4, 10 h after CS	Energetics as fresh tissue at all times for NaCl + LB + Dextran group versus decrease in energy for NaCl and UW ATP best for UW Function (permeability) better for NaCl + LB + Dextran versus UW UW and NaCl histologic damage versus intact microvilli for NaCl + LB + Dextran group	NaCl + LB + Dextran group less histologic damage and better cell function after 10 h CS as compared with UW or NaCl Osmotic and oncotic agents are a fundamental requirement for IP	Remark: All groups showed a decrease in ATP after 4 h CS
Olson, Transplantation, 2002	To test if BES (buffer) potentiates the effect of vascular GLN during IP by regulating PH IP study Spraque-Dawley rat, *N* = 16	PS groups: Vascular flush and CS UW UW + BES (UWB) UW + GLN (UWG) UW + GLN + BES (UWBG)	*In vivo* vascular flush with RPS Entire intestine retrieved CS 4, 10 h in RPS 4 °C	Parameters: Energetics (ATP/TA), barrier function, GLN catabolism, histology (light microscopy and EM) Time points: At t = 4, 10 h after CS	Increased GLN use in UWBG UWBG higher energetics after 10 h vs. other groups. Barrier better for UWBG after 10 h. CS Histologic injury + structural damage less for UWBG versus UW/ UWG	The positive effect of GLN supplementation to UW is amplified when combined with BES buffer	Strength: Addresses the metabolic principle that GLN metabolism without hepatic detoxification causes nonphysiologic PH shifts.

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Fujimoto, Am J Transpl, 2002	To assess if a luminal tailored PS protects the intestinal graft during IP IP study Spraque-Dawley rat, $N = 20$	PS groups: All groups. UW vascular flush Control: no luminal flush, CS UW Luminal flush and CS UW Luminal flush and CS UW + GLN Luminal Flush and CS AA1 (Table 2) Luminal Flush and CS AA2 (Table 2) Rats fasted	Entire intestine retrieved *In vivo* UW vascular flush Luminal flush with 20 ml RPS, distal ileum closed, infusion of 7–8 ml → CS in RPS for 1, 2, 4, 10, 24 h 4 °C	Parameters: Energetics, histology (Park score), functionality (permeability in ussing chamber with mucosa stripped) Time points: At t = 1, 2, 4, 10, 24 h after CS	All groups but UW vascular + luminal flush better energetics over 10 h vs. control/UW vascular, by 24 h no difference UW vascular + AA1/AA2 luminal better function after 10 h vs. control/UW vascular, only AA2 barrier – fresh tissue at 10 h Least damage with AA2 Good correlation Park score and other parameters	A luminal PS based on physiologic requirements (AA2: 18 AA's, BES, LB) provides targeted IP and protects intestinal graft quality AA2 showed best results Vascular PS alone is unable to protect the intestinal barrier: luminal PS decreases epithelial injury *in vitro*	Remark: Luminal nutritional support (AA) improves cellular energy and barrier function after <10 h CS, but also causes non energy-related benefit NB. Table 1 gives composition of AA solution
Leuvenink, Transpl Proc, 2005	Compare luminal PS of rat intestine with UW/CLS IP study Wag Rij rat, $N = 18$	PS groups: Luminal flush and CS UW UW + GLN CLS	Intestinal segments No vascular flush Luminal flush RPS CS for 0, 2½, 24 h in RPS 4 °C	Parameters/time points: Park score after CS, injury markers (LDH/glucose/lactate) in PS after 0, 2½, 24 h CS	Injury starts at 2½ h CS Injury markers less in CLS versus UW, but still severe histologic damage	Damage markers > for CSL luminal than UW luminal, but still severe histologic damage In addition to vascular IP alternative luminal PS is needed	Remark: Histologic damage to jejunum more than ileum

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Salehi, Transplantation, 2005	Effect of nutrient rich (amino-acid AA) luminal PS in rat ITx IP and ITx study Syngeneic orthotopic ITx model Lewis rat, $N = 15$	PS groups: All groups vascular flush UW No luminal PS UW luminal flush AA3 (Table 2 review) luminal flush Rats fasted	*In vivo* UW vascular flush Entire intestine retrieved Luminal flush according to PS groups (40 ml PS at max 40 cm H_2O) CS 6 h 4 °C NB. Vascular flush RL after CS, no luminal flush after CS	Parameters: 14-day survival, histology, energetics, tissue oxidative stress (MDA, glutathione), neutrophil recruitment (MPO) Time points: After CS, 35 min reperfusion, POD 3, 7, 14	AA3 luminal 100% 7-day and 80% 14-day survival vs. no survival >12 h for other groups. ATP levels rise after 35 min reperfusion, highest rise for AA3 luminal Oxidative stress low in AA3 group over 14 days, high in other groups. Neutrophil recruitment reduced and improved histology for AA3	AA3 luminal group showed better results on all endpoints and resulted in full regeneration of histology at POD 14: Luminal flush with nutrient rich (AA3) PS improves overall graft viability when tested in a small animal model of orthotopic ITx	
Wei, W J Gastroenterol, 2007	Compare Polysol (P) with UW/CLS/HTK for IP IP study and 30 min isolated reperfusion *in vitro* Wistar rat, $N = 28$	PS groups: Vascular, luminal flush and CS: UW CLS HTK P NB. Rats fasted 24 h	Entire small intestine Vascular flush 10 ml RPS, luminal flush saline 20 ml, RPS 15 ml CS 18 h according to luminal PS groups. 30 min *in vitro* reperfusion (in Krebs medium)	Parameters and time points: Integrity (EM), function After CS After 30 min *in vitro* reperfusion: tissue lipid peroxidation, O_2 uptake, ATP, LDH and apoptosis markers in effluent	ATP: P higher than UW/CLS, HTK higher than UW LDH release: UW less than P Peroxidation: UW less than all other groups. O_2 uptake: P better than all other groups Apoptosis: UW less than all other groups. EM: P better than all other groups.	IP with P improves graft quality on some parameters, UW shows worst trends	

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Human studies							
Olson, Transplantation, 2003	Test if results with luminal PS in rat can be reproduced in human setting + find a feasible method for luminal IP IP study Human intestine from MOD, *N* = 9	PS groups: Vascular flush UW, Intestine from 1 MOD Control: no luminal flush UW luminal flush (UWL) AA luminal flush (AAL)	Vascular *in-vivo* UW flush, Entire intestine divided in three segments for IP according to PS groups (luminal: 400 ml RPS (90 cm H_2o) + fill 50–75 ml and close) CS 24 h with RPS (as for luminal flush)	Parameters: Energetics, histology permeability (ussing chamber, NB! stripped mucosa) Time points: At 0, 4, 8, 12, 24 h CS	UWL/AAL improved barrier over 24 h UWL/AAL better histology at 12 h (24 h no difference) UWL better energetics at 24 h vs. AAL, but n.s. difference from control (UW vascular), strange!: difference from rat because full thickness samples were assessed in rat?	For human IP, AA luminal not superior to UW lum, but both luminal PS superior to UW vascular alone	Remark: Human mucosal injury became evident after 4 h CS; human intestine better/more resist ant to ischemia as compared with rat intestine
DeRoover, Trans Proc, 2004	Compare UW and CLS vascular PS for human IP IP study Human intestine from MOD, *N* = 8	PS groups: Control: UW vascular flush CLS vascular flush No luminal IP	During MOD procedure 2 ileal segments retrieved for IP according to PS groups: CS in RPS for 0, 6, 12, 24 h 4 °C	Parameters: Histologic Park score Time points: At 0, 6, 12, 24 h CS	Histology equal in difference PS Damage starts after 6 h CS at apex of villi, after 12 h epithelial sloughing, partial villous loss, finally full mucosal damage after 24 h CS	No difference for human intestinal histology after CLS/ UW vascular flush and CS over 24 h	Remark: Absent superiority of CLS also seen in experi mental setting (Minor, Transpl Int 1998)
DeRoover, Transpl Proc, 2004	Impact of luminal contact with UW during 12 h. CS on intestinal integrity IP study Human intestine from MOD, *N* = 10	PS groups: Control: UW vascular flush UW vascular + luminal flush	MOD procedure, UW vascular flush, +/– luminal UW according to PS groups. CS 0, 3, 6, 12 h with UW 4 °C Luminal UW: antimesenteric side cut open!	Parameters: Histologic Park score Time points: At 0, 3, 6, 12, 24 h CS	Control: damage starts after 3 h. CS, at 12 h villous tissue loss + detachment from BM UW vascular + luminal showed improved mucosal structure at all time points	Delayed damage pattern seen when UW luminal PS is used after UW vascular flush for human IP versus 'closed', standard CS with UW	Remark: Histologic damage starts at 3 h CS using clinical standard IP Weakness: Used method for luminal IP not clinically feasible

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Magnus, Transplantation, 2008	Compare HTK and UW for human IP + ITx Retrospective clinical review Human ITx, $N = 57$	Compares clinical results/outcome from HTK/UW preserved (vascular flush + CS) intestinal grafts	Reviews ITx and (modified) multivisceral ITx results 2003–2007 Immunosuppression: Induction: steroid, ATG, antiCD20-Ab, Maintenance: Prograft/tacrolimus	Outcome parameters: Primary outcome: graft and patient survival, early graft function, rejection episodes Also results from 2-weekly surveillance scopy + biopsy	57 ITx: 22 UW/35 HTK IP No difference in early graft function/scopy results/rejection No pancreatitis in grafts including pancreas (44) No difference in patient/graft survival at POD 30 and 90	UW and HTK show similar results of 30/ 90 day graft/patient survival, initial function, endoscopy results, rejection, or Tx pancreatitis for IP followed by clinical ITx.	Remarks: UW possibly superior for longer CS, this series max. 14 h CS Clinically better blood washout of arterioles with HTK versus UW
Perfused +/– oxygenated IP							
Toledo-Pereyra, Arch Surg, 1973	Compare oxygenated perfusion and non perfusion (CS) for IP and ITx IP and ITx study Orthotopic alloTx model Dog, $N = 24$	IP groups: Oxygenated perfusion/non perfusion (CS) IP 4 groups: Mox-100 pulsatile perfusion 24 h Mox-300 pulsatile perfusion 24 h Non perfusion Collins (CS) method 24 h 6 h CS + 18 h pulsatile perfusion Dogs fasted 5 days	Entire intestine retrieved, 24 h-perfusion groups *ex-vivo* vascular flush with RL 4 °C, other groups vascular flush with Collins IP according to IP groups. Perfusate: CPP + extra's Luminal Xylose (100 g/l) infused at start of perfusion CS: Collins PS + extra's Perfusion system: pH 7.4, 7 °C, O_2, 200 mmHg, pulse freq. 60/min, pressure 60 mmHg.	Parameters: Function restoration ITx survivors; daily exam, xylose absorption, postmortem exam Perfusate flow/pressure/ vascular resistance, fluid loss, weight gain. Perfusate: lactate, O_2 use, xylose Time points: During IP/after ITx	No difference in flow rate, perfusion pressure, vascular resistance All 6 dogs in 24 h CS/ static group died < 2 days Both 24 h perfusion groups >20 day survival: and returned to normal xylose absorption (bowel function) POD3 Clinical differences not striking: weight loss, anemia, hypo-proteinemia, anorexia, diarrhea in most dogs	24 h cold, pulsatile, oxygenated perfusion (with this perfusate) shows better outcome than 24 h CS in electrolyte (Collins) solution Possible place for perfusion IP for future longer clinical storage times	Remarks: Hemorrhagic necrosis in non perfused grafts versus no evidence of edema/ hemorrhage/ ischemia in perfusion grafts Rejection appeared several weeks after ITx

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Toledo-Pereyra, Surgery, 1974	Compare survival of fresh intestinal grafts with survival of grafts preserved by hypo-thermic bloodless perfusion IP and ITx study Orthotopic alloTx model Dog, *N* = unknown	IP groups: Control: fresh grafts/no IP Hypothermic pulsatile perfusion with canine CPP Hypothermic pulsatile perfusion with human plasmanate NB! both perfusate contain extra's	Entire intestine retrieved, luminal drainage, vascular flush with RL, followed by IP according to IP groups. Perfusion: 7 °C, pH 7.4, PO_2 200 mmHg, 60 beats/min, 60 mmHg	Parameters and time points: Perfusion: intestinal output, haemo-dynamics, composition of perfusate 2–24 h Absorptive capacity (D-xylose test/VitA). Intraluminal samples: proteins, globulin, electrolytes a 6 h Post-ITx: biopsy at reperfusion, daily clinical exam, blood sample/2 days, D-xylose + VitA test weekly. Survival	Perfusion groups no difference (O_2 use, D-xylose test) after 24 h perfusion IP, characteristics similar, fluid loss slightly more versus control Perfusion grafts histologically almost normal after IP Control group survival >2 weeks Perfusion IP grafts survival better than control, best survival with human plasmanate as perfusate.	Moderately immuno-suppressed dogs survival longer after orthotopic ITx with preserved grafts than fresh grafts; when hypothermic bloodless perfusion with canine CPP or human plasmanate is used for IP.	Suggests evidence that animals and humans possess AB against tissue antigens of xenogenic species which bind during hypothermia interfering with per ception of allogenic antigens of graft
Kuroda, Transplantation, 1996	Assess ability of the cavitary 2-layer method (C2LM) to prolong the IP time of rat intestine. IP and ITx study heterotopic segmental auto-ITx model Lewis rat, *N* = 51	IP groups: Control: no CS (i) C2LM: UW luminal + CS in PFC *with* O_2 (ii) UW luminal + CS in PFC *without* O_2 (iii) CS in UW (iv) CS in UW + O_2 Rats fasted overnight	15-cm jejunum retrieved *in vivo* vascular flush RL + heparin, luminal cleaned with RL and Gentamycin Luminal + vascular flush UW IP according to IP groups. 24, 48 h 4 °C C2LM: UW/PFC, luminal UW, graft immersed in PFC (=95%O_2 + 5%CO_2)	Parameters and time points: Daily clinical exam, 7-day survival, histology POD7	7-day survival after 24 h IP groups 1-4: 100%, 80%, 86%, 80% 7 day survival after 48 h IP 86%, 20%, 0%, 0%, most deaths caused by intraluminal bleeding/graft necrosis Histology: most grafts highly damaged from IP/IRI group 1 after 24 h IP normal mucosa, mild damage after 48 h	Oxygenation of the intestine during IP using cavitary 2-layer PFC//UW method extends IP time up to 48 h in this model	Remark: Segmental heterotopic ITx was suggested to show a different rejection pattern and will not answer the question of bowel function if recipient would be dependent on graft only.

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Tsuijmura, Am J Transplant, 2002	Assess quality of canine intestinal graft after 24 h IP using the C2LM as CS method IP and ITx study Heterotopic, segmental alloTx model Beagle dogs, $N = 28$	IP groups: Control: no CS CS with UW CS with C2LM: UW luminal + CS in PFC + O_2 Dogs fasted 24 h	40 cm jejunum segments isolated, luminal cleaned with RL + neomycin, then filled with 30 ml UW 4 °C, backtable vascular flush with RL + heparin and with UW IP according to IP groups. 24 h After IP luminal cleaned with RL, vascular flush RL + heparin	Parameters and time points: 7-day survival, graft morphology POD 7 POD7 functional analysis: maltose and acetaminophen absorption tests Histology: 1 h reperfusion and POD4	Survival: 11/12 dogs died in CS with UW group, most deaths <24 h: hemorrhagic necrosis vs. 8/8 dogs survival POD7 in C2LM group/control group. Histology: 1 h reperfusion: all groups loss of villous tissue, day 7: normal in control/C2LM group, versus loss of villous height in UW group. Absorption: control/ C2LM similar all time points, versus UW group inferior function	In this segmental canine alloTx model, the intestine was successfully preserved by CTLM for at least 24 h, while this IP time was beyond the limit with UW.	Remark: CTLM method is simple, cheap, practical, safe and without unfa vorable effect while continuously supplying O_2 to the intestine
Zhu, Transplantation, 2003	Test if oxygenated luminal perfusion (UW) facilitates energy production and preserves mucosal barrier during long IP IP study Spraque-Dawley rat, $N = 16$	IP groups: All groups vascular flush UW CS UW 24 h Luminal flush UW + CS UW 24 h 1 h luminal perfusion with UW + O_2, 23 h CS with UW 24 h luminal perfusion with UW + O_2 Rats fast overnight	Vascular *in vivo* flush 10 ml UW, entire intestine retrieved, IP according to IP groups. Luminal flush: 20 ml UW, lumen filled perfusion: after luminal flush recirculating perfusion UW (20 ml/min) + ciprofloxa cin + 100% O_2, 4 °C	Parameters: Histology, energetics, lipid peroxidation Time points: 4, 8, 12, 24 h after vascular flush	ATP levels higher in 24 h luminal perfusion group (12/ 24 h) + energy charge as fresh tissue over 24 h Lactate/ammonia less at 24 h in 24 h luminal perfusion group. MDA levels > with perfusion time Histology better in perfusion groups, best in 1 h perfusion group	Oxygenated hypothermic perfusion improves tissue energetics; however, mucosal integrity is superior with only a brief 1 h period luminal perfusion despite better energetics	Remark: Mucosal layer can only tolerate a limited period of hypother mic luminal perfusion

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Guimares, Transpl Proceedings, 2006	Assess apoptosis and nuclear proliferation in rat intestine after hypo-thermic hyperbaric oxygenated IP IP study Wistar rat, $N = 20$	IP groups: 12 h CS with RL 12 h CS with hyperbaric O_2 24 h CS with RL 24 h CS with hyperbaric O_2	*In vivo* vascular and luminal flush with? 3 cm jejunal segments preserved according to IP groups	Parameters: Immunohistochemistry: apoptotic/mitotic indices Time points: After 12/ 24 h IP	Apoptotic index higher RL groups versus hyperbaric O_2 groups. Mitotic index (nuclear proliferation) higher in group 24 h CS + hyperbaric O_2	Hypothermic hyperbaric oxygenation reduces intestinal epithelial apoptosis and increases nuclear proliferation during rat IP	Weakness: Solution of vascular and luminal *in vivo* flush is not mentioned

ATP, adenosine triphosphate; AF, alkaline phosphatase; AA, amino acid; AB, antibiotics; ATG, anti-thymocyte globulin; ADAAV, albumin-dextran-adenosine-allopurinol-verapamil solution; BES, *N*,*N*-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid; C2LM, cavitary 2-layer method; CLS, Celsior solution; CS, cold storage; CP, creatine phosphate; CPP, cryoprecipitated plasma; EM, electron microscopy; EC, EuroCollins solution; ECF, extracellular fluid; GLN, glutamine; HTK, histidine tryptophan ketoglutarate; IP, intestinal preservation; ITx, intestinal transplantation; i.p., intra-peritoneal; IRI, ischemia reperfusion injury; LB, lactobionate; RL, lactated Ringer's solution; LDH, lactate dehydrogenase; LF, lactobionate fructose; LF2, lactobionate fructose 2; LPO, lipid peroxidase; MDA, malonaldehyde; MPO, myeloperoxidase; NEAA, nonessential amino acids; n.s., nonsignificant; MOD, multi organ donor; PBS, phosphate buffered sucrose; PC, preservation conditions; POD, postoperative day; PD, potential difference; PS, preservation solution; ROS, reactive oxygen species; R, resistance; RPS, respective preservation solution; S, saline; SGOT = AST = ALAT, serum glutamic oxaloacetic transaminase; SGPT = ALT = ALAT, serum glutamic pyruvic transaminase; TA, total adenylates; Tx, transplantation; UW, University of Wisconsin Solution; versus = compared to (vs.).

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clinically used solutions contains PEG. Wei *et al.* [25] suggested that the low viscosity resulting from replacing HES with PEG in Polysol was partly responsible for the superiority of Polysol over UW. This is plausible, as simple crystalloid solutions earlier demonstrated a better wash-out and reperfusion characteristics than UW [24,26]. On the other hand, colloids are important *during CS* [39] to minimize cellular edema and therefore are to be included.

The clearly demonstrated sequential pattern of morphologic damage to the intestinal graft is caused by edema [40]. Mucosal damage starts with the formation of sub-epithelial clefts at the villus tip, followed by epithelial lifting from the lamina propria along the villus, progressive denudation, loss of villi and finally mucosal infarction. Morphologic damage negatively affects the protective barrier and absorptive capacity of the intestine.

The necessity for oncotic impermeant support, particularly during IP, was investigated for prolonged (10 h) CS of rat intestine [41]. The intestinal vasculature was flushed with one of the four solutions: (i) saline (0.9%), (ii) saline + 5% dextran, (iii) saline + lactobionate + dextran or (iv) UW. Cellular energy, function (permeability) and histology were analysed during a 10-h time course of CS. Saline + lactobionate + dextran resulted in better maintenance of energy levels and improved function (versus UW) in combination with intact morphology, versus extensive villus denudation and loss of crypt cells in saline and UW. Noteworthy, as crypt cells carry the regenerative capacity. These results confirm the importance of osmotic and oncotic impermeant support during IP and may imply that dextran might be a superior alternative for the colloid HES in UW. Unfortunately, wash-out, reperfusion characteristics and ITx outcome were not assessed. In contrast, Rodriguez *et al.* [42] showed that albumin-dextran-allopurinol-adenosine-verapamil (ADAAV) preservation solution, also containing albumin and glucose, was not superior to UW (HES, lactobionate, raffinose) or Euro Collins (glucose) for rat IP. Outcome was assessed after 24-h CS and ITx. Biochemical and histologic parameters did not differ among preservation groups. These results suggest the crucial role of lactobionate as the superiority of dextran in combination with lactobionate has been demonstrated [40].

Impermeants and colloids are key factors to counteract deteriorating fluid shifts during intestinal preservation [40]. Dextrans or PEG might be superior colloid alternatives for HES with better wash-out and reperfusion characteristics [25,26,41]. Substantial evidence exists to suggest that lactobionate is important for effective impermeant support.

Buffering capacity

Ischemia results in anaerobic glycolysis and glycogenolysis. These anaerobic processes produce lactic acid and hydrogen ions resulting in acidosis, which subsequently damages cells, lysosymes, and mitochondria. Prevention of tissue acidosis is therefore an important prerequisite for good organ preservation.

A variety of buffers are applied to regulate pH homeostasis during preservation: UW relies on a phosphate buffer, HTK is based on histidine, while Polysol contains a phosphate buffer, histidine and the sulfonic buffer HEPES. The exact value of buffer type in the preservation solution regarding intestinal viability after CS cannot be determined as no single study has compared solutions and assessed pH homeostasis in relation to ITx outcome.

The physiologic buffering agent histidine has been related to minimized pH fluctuations during CS [43]. The buffering potential of histidine was investigated for IP particularly [44]. Histidine supplementation to UW resulted in a greater than threefold increase in buffering capacity (pH range 7.4–6.8) and enhanced glycolytic capacity with a higher value of ATP and total energy charge during 10-h CS of rat intestine. Improved energy levels were attributed to activation of the key enzyme phosphofructokinase (PFK) and alleviation of intracellular acidosis in the presence of histidine. Although these findings give valuable insight into biochemical processes during IP, histology, functionality and outcome after ITx were not studied.

Anti-oxidants

Upon reperfusion, accumulated anaerobic end-products contribute to the generation of ROS. These ROS will severely damage lipids, nucleic acids and proteins and provoke a profound inflammatory response. It has also been suggested that ROS are already generated during CS. To reduce injury, preservation solutions contain variable ROS scavengers to counteract ROS-mediated injury during CS and reperfusion.

University of Wisconsin Solution includes allopurinol and reduced glutathione to counteract the effect of ROS; in Celsior just glutathione is present. Allopurinol blocks xanthine oxidase, whereas the tripeptide glutathione converts peroxides as it is oxidized. Minor *et al.* [20] demonstrated that neither glutathione (in Celsior), nor the combination of glutathione and allopurinol (in UW) prevent oxidative stress in rat intestine after 18 h of CS and *in vitro* reperfusion.

In HTK, tryptophan functions as a ROS-scavenger by its oxidative (electron-accepting) metabolites. Wei *et al.* [25] reported that 18 h of CS of rat intestine with HTK, CLS or Polysol resulted in less peroxidation than with UW after 30 min of *in vitro* reperfusion as malondialdehyde was lower in Polysol. In addition, Polysol exhibited the highest ATP concentrations and least apoptosis, versus the lowest ATP concentrations and highest apoptosis

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with UW solution. The favorable results with Polysol could be in part explained by an optimized 'anti-oxidative potential' resulting from supplementation with a broad variety of ROS-scavengers (Table 1). It appears that anti-oxidative ability attenuates damage through improved energetics and reduced apoptosis. Celsior and HTK (Table 1) both contain relatively high concentrations of histidine, which is also suggested to neutralize ROS. The protective capabilities of L-histidine were specifically linked to the ability to scavenge toxic ROS [45]. Possibly, the low concentrations of allopurinol and glutathione in UW provide 'marginal oxidative potential', which might explain increased tissue oxidation for UW.

In conclusion, low concentrations of allopurinol and glutathione (as in UW) do not entirely prevent oxidative stress [20]. High concentrations of histidine in addition to glutathione/tryptophan or a combination of antioxidants as used in Polysol can attenuate oxidative stress and benefit intestinal graft quality.

Amino acid supplementation

Following preservation and at times of reperfusion, the presence of substrate to rapidly regenerate cellular energy is crucial. To facilitate this process ATP precursors such as AA are added during IP in order to improve viability. Hypothermia causes rapid degradation of high-energy compounds [5,46]. Energetic status is related to metabolic cell stress, histologic damage and apoptosis, all affecting structural and functional integrity [25,47]. At the ultrastructural level of intestinal tissue, TJ depend on ATP. Depletion of energy leads to delocalization and degradation of TJ resulting in increased epithelial permeability and influx of macromolecules [48]. Upon energetic restoration, fortunately, TJ re-assemble and barrier function is restored.

Amino-acids are postulated to play a protective role by their facilitation of metabolic and synthetic cellular processes during preservation [43,49–51]. Nevertheless, Olson *et al.* [41] demonstrated that maintenance of mucosal structural integrity with AA supplementation may not be energy-dependent but substrate-specific, as AA solution favored histology, while UW resulted in higher energy levels. Energy levels should therefore be interpreted carefully when used as a solitary parameter of viability.

Especially glutamine, the principle energy substrate for the enterocyte, is suggested to be beneficial. Glutamine-supplied RL was shown to be beneficial for mucosal cell structure during IP as compared with RL. Vascular glutamine supply merely favored crypt cells, whereas luminal supply ameliorated cells at the tip of the villi [14]. In line, glutamine-enriched UW improved rat mucosal function and structure of after 18 h CS [52]. Upon comparison of different glutamine concentrations and other AA (2–4%), the solitary addition of 2% glutamine to UW solution was most effective. Olson *et al.* [53] addressed a potential danger of detrimental pH-shifts when glutamine metabolism is sustained in a system devoid of hepatic detoxification. Specifically, acidic TCA-cycle related end-products like ammonia must be buffered. This possibly explains why 4% glutamine was not superior to 2% glutamine. Considering AA supplementation in general, only the 4% AA concentration showed better results than UW, perhaps indicating a threshold to provide energy stores.

The superiority of Polysol over UW (and over Celsior and HTK on some endpoints) has been attributed to the variety of AA (Table 1) in Polysol [25]. Recapitulating, Polysol resulted in higher ATP levels, lower LDH production, higher oxygen consumption and better preserved microstructures as compared with UW. Although a benefit of the extensively enriched Polysol was reported, the exact mechanisms and the separate values of the 21 different AA compounds were not clarified. Noteworthy, in the latter study both a *vascular flush and a luminal flush* with the different solutions were applied.

In summary, 4% AA or 2% glutamine supplementation to the preservation solution in the presence of sufficient buffering capacity has a potential to ameliorate rodent intestinal graft quality. The mechanisms are to be elucidated more extensively in ITx models and in human IP studies.

Preservation conditions

Intravascular preservation

A standard, high-volume, *in situ*, systemic wash-out before CS is applied for rapid blood clearance and temperature decrease of the donor organ. In contrast to most solid organs, the intestine lacks a supportive capsule and might be unable to withstand strong mechanical forces leading to edema and mucosal detachment. Consequently, the intestine is recovered first after a limited volume of systemic flush [54]. The precise volume or pressure not to 'overstretch the mechanical endurance' of the intestine is unknown yet. Short-term (1 h) pulsatile perfusion at a pressure of 60 mmHg in combination with CS appears to be superior to only CS for 24-h graft preservation in the dog model, as described in the last paragraph of this review.

The value of the first vascular flush before CS was evaluated over 24-h CS and ITx in the rat [42]. Rat intestine was preserved with different solutions directly after recovery or after a vascular flush *ex situ*. Outcome was based on biochemistry, histology, functional glucose uptake, survival and determinants of failure. Survival was 0%

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without a vascular flush before CS, whereas 47–67% survival in the flushed groups.

After CS, a second vascular flush has been considered harmful. Muller *et al.* assessed survival, histology and glutaminase activity after 12 h with and without a *second* vascular flush after CS [26]. Also, the effect of intraperitoneal abdominal rewarming upon reperfusion was studied out of concern for insufficient blood supply after CS. Survival was highest when a second vascular washout was omitted and re-warming with 37 °C saline intraperitoneal was applied. A second vascular flush after CS appeared to cause substantial mechanical damage, overruling any theoretical advantage of removing accumulated toxic components.

Thus, a vascular (*in situ*) wash-out before CS appears to be critical and a vascular flush after CS should be omitted. Topical abdominal rewarming during reperfusion may deserve further evaluation.

Intraluminal preservation

In addition to the intra- and extra-vascular compartments, the intestinal lumen represents an additional space of interest. Tissue edema is believed to originate from the lumen along with increased permeability during ischemic preservation [55]. Furthermore, it is potentially contaminated by bacteria. Finally, its surface volume and the susceptible epithelial top layer underline a role for the lumen. These characteristics possibly explain why a vascular wash-out and CS are unable to support the intestinal graft sufficiently. As highly vulnerable epithelial cells at the villus apex rather rely on nutrient absorption from the lumen than vasculature, [14,56] the lumen forms a logical route to improve preservation conditions.

A benefit of luminal preservation (before CS) was originally explained through clearance/dilution of resident enteric cytotoxic contents [57,58]. Luminal preservation with a crystalloid solution ameliorated mucosal function as compared with vascular preservation alone, but the benefit was at the expense of morphologic integrity [59]. Luminal preservation with nutritive substances was another conceived strategy. Continuous luminal and/or arterial perfusion (24 h) of canine jejunum with glutamine-enriched RL suppressed preservation injury and improved function and structure as compared with perfusion with just RL [14]. The combination of luminal and arterial perfusion with glutamine-enriched Ringers' Lactate (RL) prevented cell damage caused by energetic deficiency, and favorably maintained the number of epithelial cells, viability, and protein metabolism. Yet, a benefit of glutamine-enriched luminal flush and CS (24 h) of rat intestine could not be shown by Leuvenink *et al.* [60] when comparing Celsior, UW, and glutamine-enriched UW. As regards the biochemical endpoints, Celsior seemed to be the best luminal preservation solution. However, histology showed severe damage in all groups. As a vascular wash-out before CS was omitted, histologic findings might again confirm the critical role of this element.

deRoover *et al.* evaluated luminal preservation with UW (after vascular wash-out) on *human* intestinal histology [61]. Luminally preserved segments showed less histologic damage than standard-preserved intestinal segments from the same donor after 12 h CS. Functionality and outcome after reperfusion were not studied.

Further studies were directed at improvement of graft viability by tailoring luminal solutions to support energy and nonenergy-related processes. Luminal exposure to AA was held responsible for the overall superiority of Polysol as compared with UW [25] when applying vascular and luminal flush with different solutions before CS. In an attempt to develop the optimal tailored luminal solution, Fujimoto *et al.* [62] compared luminal preservation (after UW vascular flush) and 24 h CS of rat intestine with four different luminal solutions [UW, glutamine-enriched UW, and two similar AA (Table 1) solutions] to the clinical standard of vascular wash-out alone followed by CS (control). All enriched luminal groups showed improved functionality, energetics, and histology as compared with the control group. Best functionality and morphology were seen after luminal preservation with the AA solution including lactobionate and *N*,*N*-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) buffer. Outcome after ITx must prove if the luminal benefit is substantial enough to withstand the negative effects of reperfusion.

Salehi *et al.* [63] attempted to further clarify the potential beneficial mechanisms of luminal AA-enriched IP. Luminal flush and exposure during CS with AA-enriched solution (after vascular UW flush) resulted in recovery of energetics within post-transplant day 3 and reduced malondialdehyde/glutathione, indicating less oxidation following reperfusion. The reduction in energetic- and oxidative stress was likely responsible for a simultaneous decrease in neutrophil recruitment and histologic damage in grafts after luminal AA preservation. AA luminal preservation resulted in a 14-day survival of 80%, while all animals died within 12 h after preservation with UW (vascular/luminal flush).

Despite a benefit of luminal AA-enriched preservation solution over luminal standard UW in rodent studies, this was not seen for human intestine. The effect of a luminal flush with AA solution or UW (after UW vascular wash-out) on outcome of human intestine over 24-h preservation was assessed [64]. Luminal AA did not prevent ATP decay any better than isolated vascular UW solution. Both luminal preservation groups resulted in better barrier

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function and morphology as compared with standard preservation.

Recently, Oltean *et al.* [65] reported that intraluminal preservation (after UW vascular wash-out) with a low-sodium, PEG-containing solution ameliorated preservation damage in rat intestine. Luminal preservation improved morphology and reduced edema after 14-h CS as compared with a vascular flush alone. Luminal PEG was suggested to have maintained epithelial integrity by its ability to bind to negatively charged sphingolipids on the enterocytes. As luminal preservation with UW was not assessed, the possibility of an aspecific luminal effect remains.

The benefit of luminal preservation of rodent and human intestinal grafts has been confirmed, but AA-enriched luminal preservation showed no additional advantage (over UW) for preservation of human intestine. Furthermore, outcome after human ITx must ultimately prove if a luminal benefit can reduce reperfusion injury.

Hypothermic perfused preservation and oxygenated techniques

Hypothermic machine perfusion (HMP) generates a flow of recirculating cold preservation solution. For the intestine, CS is assumed to be superior to HMP on grounds of apprehensions over possible pressure-induced vascular injury. However, the comparison of two pulsatile perfusion systems with CS using Collins solution and with a combination of 6-h CS + 18-h pulsatile perfusion demonstrated a better outcome after machine perfusion preservation (at 60 mmHg) in the dog [66]. Significant differences were noted between CS and the combined technique, suggesting that initial pulsatile perfusion may be decisive.

Hypothermic oxygenated luminal perfusion – simultaneously delivering oxygen luminally and removing toxic products – also ameliorated viability [66]. Despite improved energy levels (ATP) and decreased lactate/ammonia after 24-h perfusion, histology was superior with only a 1-h period of luminal perfusion. This reconfirms the limited value of energetics, and indicates that the intestine tolerates a limited period of hypothermic luminal perfusion. Mechanical disruption is likely to be responsible for the histologic damage. Perhaps, the intestine could profit from metabolic benefits with a different perfusion technique. Alternatively, normothermic perfusion may be advantageous. Alterations in membrane fluidity may be less problematic in this situation, but bacterial overgrowth becomes another concern. Normothermic oxygenated machine-perfusion (NMP) supports normal metabolism and minimizes the accumulation of ROS precursor-substrates. Improved canine/rodent intestinal graft quality and longer storage spans have been reported during 1960–1970s [67] using complicated techniques including continuous perfusion and hyperbaric oxygenation. ITx was successful after 5 h of NMP with heparinised RL [68]. Pulsatile perfusion with whole blood at 37 °C maintained intestinal graft viability for 18 h (*in vitro*). When nonpulsatile flow was used, the graft survived only 6 h [69].

Several intricate oxygenation techniques have been attempted. A static, hypothermic, cavitary, two-layer-oxygenated perfluorcarbon/UW method (cavitary 2-layer method, C2LM) was evaluated for its ability to prolong preservation times of rat intestine in a transplant model [70,71]. Survival and graft histology was compared after C2LM ± oxygen or hypothermic preservation with UW ± oxygen for 24/48 h. The C2LM (-oxygen) allowed preservation for 24 h. With oxygen, preservation span was expanded up to 48 h. However, UW + oxygen was not effective without PFC, indicating the necessity of a high oxygen-tension carrier. Tsujimura *et al.* [70,72] evaluated the C2LM for *canine* preservation and ITx. All dogs in the C2LM group survived, while 11 of 12 dogs in the UW (static CS) group died. Graft histology and absorption capacity in the C2LM group was similar to nonpreserved grafts.

Normothermic oxygenated (luminal) preservation facilitates physiologic metabolism of the intestine by maintaining energy stores/ reducing oxidative stress and removal of toxic products. If a delicate technique can be developed, a role for normothermic oxygenated (luminal) preservation is conceivable.

Conclusion

Improvement of intestinal preservation is a subject of great interest as reduced graft quality is recognized to limit the outcome of ITx. Apart from immunosuppressive interventions, advancement should be directed towards new preservation strategies. The current preservation regime with UW is adequate but probably suboptimal for intestinal preservation. Study results have been mostly incomparable because of a wide heterogeneity of species, experimental set-up, and outcome parameters. Most studies have been on animals, while human studies have been scarce. Furthermore, the compared solutions often have many different components and any beneficial outcome is therefore not easily explained. Finally, inconsistency among parameters occurs, questioning the validity of different parameters. Functional parameters seem preferable: however, histologic crypt status is critical for estimating the reparative capacity.

Although available studies do not reveal the most effective technique and solution for intestinal preservation,

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Table 3. Lessons learned regarding intestinal preservation.

Lessons learned from animal studies *Beneficial strategies*	LOE	Lessons learned from human studies *Beneficial strategies*	LOE
• Vascular flush before CS • No 2nd vascular flush after CS • Topical intra-peritoneal rewarming during reperfusion • Luminal contact during preservation • Amino-acid supplementation in vascular/luminal preservation solution • Vascular/luminal supplied colloid and impermeant support • Buffering capacity of the preservation solution • Hypothermic perfused preservation with blood-like solutions • Luminal oxygenated perfusion for a short period • Normothermic oxygenated perfusion • Assess viability based on combination of functional, biochemical, and histological parameters	Low: Animal studies	• Luminal preservation: aspecific dilution/cytoprotective contact between mucosa and preservation solution	3
Harmful strategies		*Harmful strategies*	
• Fasting of the donor • Applying a second vascular flush after CS	Low: Animal studies		
General pitfalls		*Future considerations*	
• Lack of a clinical applicable method for luminal preservation • Functional and biochemical parameters affected before histology and possibly more sensitive than histology for graft viability assessment • Lack of practical functional/biochemical parameters to assess intestinal viability • Lack of ITx as the primary outcome parameter		• Similar short-time clinical outcome of ITx after preservation with UW/HTK • Potential superiority of HTK over-shadowed by limited preservation span of intestine • HTK less expensive and better wash-out of blood and reperfusion characteristics • Luminal tailored preservation • Normothermic (perfused) oxygenated techniques	

CS, cold storage; HTK, histidine tryptophan ketoglutarate solution; LOE, level of evidence; UW, University of Wisconsin solution.

this review illustrates that alternative strategies can improve graft quality and outcome after ITx. The intended optimal strategy will not rely on just one factor, but will result from a synergistic effect of different vital elements within a 'package of conditions' (Table 3). A vascular flush before CS cannot be omitted. A low potassium/low viscosity solution without HES allows for better washout of blood than UW. AA offer advantages to viability. Osmotic, oncotic (e.g. PEG, lactobionate and raffinose) and buffering agents are fundamental in a preservation solution. There is a benefit of luminal flush and/or contact between the mucosa and the solution during preservation, although the best composition of the luminal solution for the human intestine and a practical, clinically applicable optimal technique are yet unknown.

Oxygenated arterial and/or luminal perfusion for short periods are to be considered, as this technique maintains viability and additionally removes accumulated toxic products. Powerful organic oxygen carriers, allowing for high oxygen-tension, can be applied.

Thus, a tailor-made luminal preservation solution and/or (oxygenated perfused) technique need to be further investigated for the human setting and in transplant models to develop the ultimate technique that meets physiologic demands of the intestinal graft during preservation.

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