Transpl Int (1998) 11: 32–37

ORIGINAL ARTICLE

T. Minor
B. Vollmar
M.D. Menger
W. Isselhard

Cold preservation of the small intestine with the new Celsior-solution

First experimental results

Received: 13 June 1997
Received after revision: 19 September 1997
Accepted: 8 October 1997

T. Minor (✉)
Department of Surgery,
Surgical Research Division,
University of Bonn,
Sigmund-Freud-Strasse 25,
D-53127 Bonn, Germany

W. Isselhard
Institute for Experimental Medicine,
University of Cologne,
Robert Koch Str. 10,
D-50931 Cologne, Germany
Fax: +49 221 478 62 64

B. Vollmar · M. D. Menger
Institute for Clinical-Experimental
Surgery, University of Saarland,
D-66421 Homburg/Saar, Germany

Abstract The aim of the present study was to evaluate the potential of Celsior, a recently developed cardioplegic and heart storage solution, to protect the small bowel during ischemic storage. Small bowel segments were isolated from rats, flushed with either UW or Celsior solution, and cold-stored for 18 h at 4 °C in the respective solution. After ischemic storage, some preparations were freeze-clamped for analysis of tissue metabolites while other preparations were tested for structural and functional integrity by isolated perfusion in vitro using a previously validated model. After 18 h of ischemic storage no significant differences were seen between Celsior and UW with regard to the development of edema, energy charge, or creatine phosphate, but lactate accumulation was significantly reduced in the Celsior group, although glucose catabolism was not inhibited. Histological evaluation of the cold-stored organs showed no differences with regard to structural integrity between the two groups. Total vascular resistance upon reperfusion was significantly lower in the Celsior group (666 ± 126 vs 827 ± 88 MPa s m^{-3}*), as was the intestinal release of LDH (9.7 ± 4.4 vs 18.2 ± 4.6 U/l *). Carbohydrate absorption from the intestinal lumen amounted to venous effluent concentrations of 0.58 ± 0.24 vs 0.18 ± 0.15 mg% * of galactose in the Celsior and UW groups, respectively. Within the limits of this in vitro pilot study, Celsior provided better postischemic recovery of the small bowel than UW in terms of vascular perfusion characteristics, enzyme release, and carbohydrate absorption and may, thus, be considered a suitable alternative for intestinal organ preservation.

Key words Celsior, preservation · Small bowel, preservation · Preservation, small bowel

Introduction

Celsior is a recently developed, extracellular type, low viscosity preservation solution (Table 1) that has been shown to have beneficial effects on heart [14] and lung [2] preservation. The small intestine is known to be particularly susceptible to preservation/reperfusion injury and the preservation solution of choice for a small bowel graft is still controversial [5]. Therefore, much effort has recently been put into evaluating and improving methods for long-term preservation of the small bowel [13, 24, 27]. Yet, tissue injury related to ischemic preservation of the intestine, with ensuing destruction of the mucosal cell layer may still seriously compromise the viability of the organ upon transplantation [9, 11].

The aim of the present study was to evaluate the potential of Celsior to protect the small bowel during ischemic storage by comparing the metabolic status and functional integrity of isolated intestinal segments after ischemic preservation in either Celsior or University of

Table 1 Composition of Celsior in comparison to UW preservation solution (mmol/l)

	UW	Celsior
Na^+	30	100
K^+	125	15
Mg^{++}	5	13
Ca^{++}	–	0.25
SO_4^{--}	5	–
PO_4^-	25	–
Mannitol	–	60
Histidine	–	30
Lactobionate	100	80
HAES	50 g/l	–
Glutamic acid	–	20
Adenosine	5	–
Allopurinol	1	–
Glutathione	3	3
Dexamethasone	8 mg/l	–
Insulin	100 U/l	–
Viscosity	4.8 cP	1.3 cP

Wisconsin (UW) solution, a solution that has been shown to maintain viability of bowel grafts for up to 16 h of storage in humans [3].

Materials and methods

All experiments were performed in accordance with the federal law regarding the protection of animals. The principles of laboratory animal care (NIH Publication No. 85–23, revised 1985) were followed.

Male Wistar rats weighing between 225 and 275 g were anesthetized by intramuscular injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). Atropine (0.05 mg/kg) was given to reduce salivation of the animals. During the operation, oxygen was given through a nasal catheter in order to avoid the development of hypoxia.

The abdomen was opened by a midline incision with bilateral subcostal extensions, and a 15-cm segment of the upper jejunum was isolated with the vascular pedicle, as described elsewhere [7, 18]. The superior mesenteric artery was cannulated with PE tubing (0.5 × 0.9 mm) and the jejunal segment was then flushed with 10 ml UW or Celsior preservation solution at 4 °C. The portal vein was cannulated with a large PE splint (ID 15 mm), later connected to silicone tubing to allow for collection of the venous effluent upon reperfusion. A short PE cannula (14 G) was inserted into the upper jejunal lumen and secured with a circumferential tie. The intestinal lumen was gently irrigated with 10–15 ml of the respective preservation solution.

The segments were excised and stored ischemically for 18 h in a bath of 100 ml UW or Celsior solution. The temperature was kept between 2 ° and 4 °C by means of an external cooling circuit (Ministat digital thermostat, Fa. Huber Offenburg-Eigersweier). At the end of ischemic preservation, specimens from each group (n = 5–7) were snap-frozen in liquid nitrogen and preserved below –40 °C until later analysis. Control values for tissue biochemistry were obtained from control samples harvested from vital intestines in vivo.

Other preparations (n = 6) were reperfused in vitro after ischemic preservation according to previously established techniques [18] in a non-recirculating fashion at a flow rate of 5 ml/min. The preparations were initially rinsed with 5 ml of NaCl 0.9 % via the superior mesenteric artery and then placed into a temperature controlled organ bath of 37 °C NaCl 0.9 %. The perfusate consisted of a modified Krebs-Henseleit buffer, supplemented with 5 % dextran 78 [18] and oxygenated with 95 % O_2 and 5 % CO_2 The temperature of the perfusate was kept at 37 °C. The intestinal lumen was perfused at a rate of 0.5 ml/min with saline solution containing 200 mg% of galactose.

Metabolic analysis

In liquid nitrogen the frozen intestines were freed from surrounding tissue and frozen water using a dental drill. The specimens were then weighed and transferred into a vacuum freezer. After tissue water was evaporated during at least 5 days of freeze drying, the samples were weighed again to determine the total dry weight of the specimen, and tissue concentrations of adenine nucleotides, creatine phosphate, glucose, and lactate were determined by standard enzymatic tests, as described previously [8, 12]. As a reference, metabolites were also measured in intestinal segments harvested from normal intestine in situ and processed as described above. The energy charge potential (ECP) was calculated according to Atkinson [1] as: ECP = (ATP + 1/2 ADP)/(ATP + ADP + AMP).

Histology

Specimens were rapidly fixed in 10 % formaldehyde and embedded in paraffin. Three-micrometer sections were stained with hematoxylin-eosin for routine histology.

Vascular resistance upon reperfusion

The mean arterial pressure during isolated perfusion via the superior mesenteric artery was measured continuously by means of a precalibrated Stadham (P 23) pressure transducer (Gould, Oxnard, CA) connected to the arterial inflow line. The total vascular resistance (TVR) of the preparations was then calculated according to the following formula [15]:

TVR (GPa s m^{-3}) = MAP (mmHg)/Q (ml/min) × 7.998, where MAP represents the mean arterial pressure and Q the arterial inflow.

Release of lactate dehydrogenase (LDH)

Effluent activities of LDH were determined at definite time points, i.e., after 2, 15, and 30 min cf reperfusion from aliquots of 0.1 ml using commercialized photometric test kits (Boehringer, Mannheim).

Release of lipid peroxidation products

Lipid peroxides (LPO) were measured in the portal effluent using HPLC techniques described earlier [16, 26] and taken as a parameter to approximate oxygen free radical-induced tissue injury upon oxygenated reperfusion of the small bowel grafts.

Table 2 Metabolic status of small intestines after 18 h of ischemic preservation at 4 °C in Celsior or UW solution in comparison to nonischemic control tissue. Data are given as means ± SD of 5--7 observations (*ECP* energy charge potential, *CP* creatine phosphate, *dw* dry weight)

	ECP	CP (μmol/g dw)	Glucose (μmol/g dw)	Lactate (μmol/g dw)	Dry weight (% of wet weight)
Control	0.71 ± 0.09	5.13 ± 0.80	37.3 ± 8.5	6.2 ± 1.4	21.8 ± 3.9
18 h UW	0.35 ± 0.04*	0.25 ± 0.19*	13.8 ± 8.2*	39.0 ± 8.2*	21.5 ± 2.4
18 h Celsior	0.42 ± 0.15*	0.50 ± 0.18*	5.5 ± 5.2*, **	6.6 ± 1.2**	20.6 ± 3.1

* $P < 0.05$ vs control; ** $P < 0.05$ vs UW

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 from the intestinal lumen may be considered a useful parameter to approximate mucosal carbohydrate absorption.

Transcapillary fluid loss

Net movement of water from the vascular compartment into the intestinal lumen was calculated after quantitative measurement of the luminal effluent during postischemic reperfusion of the preparations.

Statistics

All results are expressed as means ± standard errors if not otherwise indicated. Comparisons between multiple groups were performed with Dunn's test after one-way analysis of variance (ANOVA). Nonparametric analysis of differences between two groups was performed using the Mann-Whitney U-test. Differences were considered statistically significant when P was below 0.05.

Results

Metabolic status after ischemic preservation:

At the end of ischemic preservation, the intestinal energy charge potential was found ti have decreased to about half the values observed in controls in vivo, irrespective of the preservation medium used (Table 2). Accordingly, a significant loss of creatine phosphate was evident after 18 h of preservation in UW; similar results were seen after preservation in Celsior.

We found considerably more inhibition of lactate accumulation during ischemia in the Celsior group than in the UW group. In contrast, glucose catabolism did not seem to be inhibited during preservation with Celsior since tissue levels of glucose were rather low and significantly inferior to those observed after preservation with UW (Table 2).

Tissue edema was expressed as the percentage of dry weight to wet weight of the intestinal preparations. It can be seen in Table 2 that each of the preservation solutions was adequate in terms of preventing an increase in tissue water content during the 18 h of ischemic storage.

Histological evaluation of the cold-stored organs showed an equally well-preserved structural integrity in both groups at the end of the cold preservation when compared to the nonischemic controls (Fig. 1).

Functional evaluation of small bowel preparations after ischemic preservation

Upon postischemic reperfusion, the total vascular resistance (TVR) was found to be constant during 30 min of perfusion in nonischemic controls. Preservation with UW resulted in a progressive and significant rise in vascular resistance with respect to the controls, while the values remained significantly lower and near the normal range in the Celsior group (Fig. 2).

In the control group, most of the preparations were able to absorb water from the intestinal lumen, even though only small amounts were transported under the experimental conditions. After cold storage of the bowel segments, transcapillary loss of fluid was seen in both groups, irrespective of the preservation solution used (Table 3).

Intestinal carbohydrate absorption, i.e., in our model, transportation of galactose from the intestinal lumen into the vascular system, was measured as the concentration of galactose in the venous effluent. Ischemic preservation led to a significant deterioration of mucosal absorption upon immediate reperfusion. However, a notable improvement could be achieved using Celsior instead of UW solution.

LDH was also assessed in the perfusate and taken as a general parameter of cellular injury of the preparations. While nonischemic controls showed only minor enzyme activity in the effluent, a significant elevation in LDH release was evident upon reperfusion of intestines stored in UW, with up to twice the values observed in controls. Preservation of the small bowel with Celsior resulted in a significant reduction in LDH release upon reperfusion.

The impact of oxygen free radicals at the time of reperfusion was assessed by measuring lipid peroxides

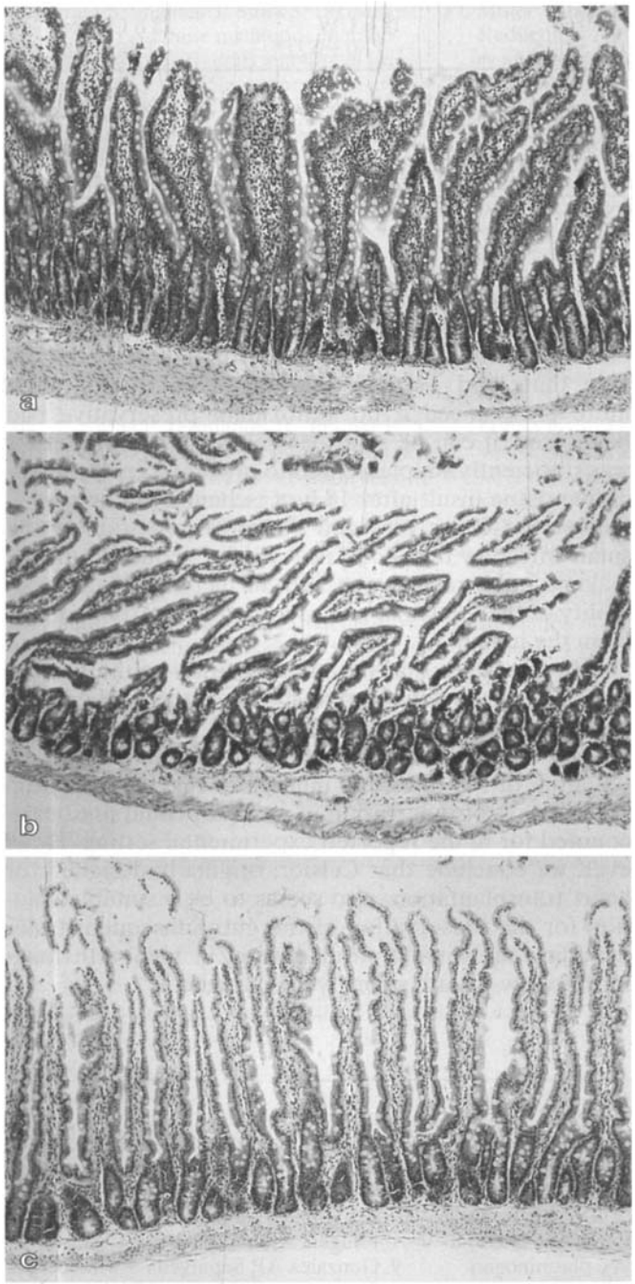

Fig. 1 a–c Histologic appearance of rat small intestines: **a** in vivo and after 18 h of hypothermic ischemic preservation in **b** Celsior or **c** UW solution. (H&E, × 93)

(LPO) released into the perfusate. The maximal release of LPO was significantly elevated after preservation in UW or Celsior compared to the nonischemic controls, but no differences were seen between the two preservation solutions.

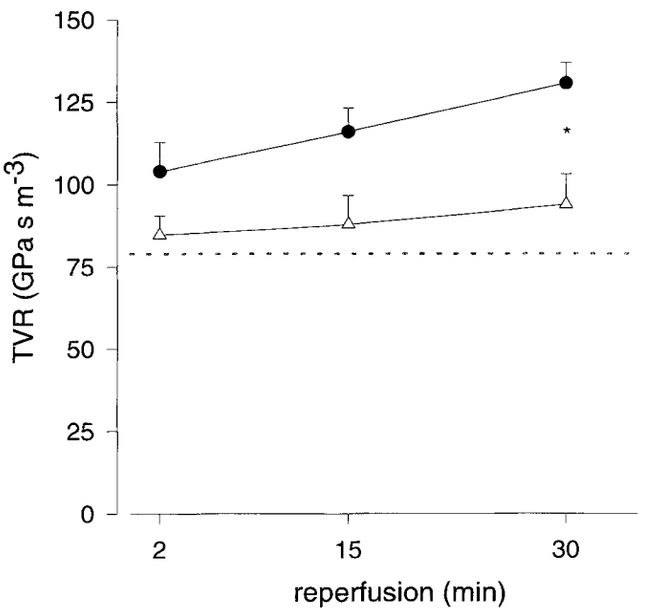

Fig. 2 Total vascular resistance (*TVR*) of the isolated intestinal preparations upon perfusion via the superior mesenteric artery in vitro after 18 h of hypothermic preservation in UW (●) or Celsior (△) solution. The *hatched line* represents reference values obtained by perfusion of nonischemic control preparations. Values given represent mean ± SEM of five or more observations. * $P < 0.05$ vs UW

Discussion

We have investigated the effects of hypothermic storage of small bowel segments on metabolic status during ischemia as well as the functional integrity of the preparations thereafter. The main objective of this study was to give a primary evaluation of Celsior in the new setting of intestinal organ preservation. We were able to show that Celsior protected the ischemic intestine as well as, and often even better than, UW solution.

The degree of degradation of high-energy phosphates in the cold-preserved small bowel specimens observed in our study was comparable to data reported in the literature after intestinal storage in UW solution [4, 22]. There were no differences between Celsior and UW with regard to the energy charge potential at the end of ischemia. However, a pronounced depletion of intestinal glucose content was evident in the presence of Celsior. The biochemical factors responsible for the increased glucose utilization under anaerobic conditions with Celsior cannot be established based on the results of the present study. It has been reported that glutamic acid, present in Celsior but not in UW or other preservation solutions, might augment energy production under anaerobic conditions [21]. However, in our model, the addition of glutamic acid to UW had no major influence

Table 3 Functional recovery of intestinal preparations upon reperfusion after 18 h of ischemic preservation at 4 °C in Celsior or UW solution. Data are given as means ± SD of 5–7 observations (*LDH* lactate dehydrogenase, *LPO* lipid peroxides)

Water balance (ml/min)	Control 0.01 ± 0.15	18 h UW − 1.50 ± 0.21*	18 h Celsior − 1.48 ± 0.43*
Carbohydrate absorption (mg % galactose)	1.97 ± 0.48	0.18 ± 0.15*	0.58 ± 0.24*,**
LDH_{max} (U/l)	9.6 ± 2.1	18.2 ± 4.6*	9.7 ± 4.4**
LPO_{max} (pmol/ml)	627 ± 154	892 ± 92*	868 ± 56*

* $P < 0.05$ vs control; ** $P < 0.05$ vs UW

on end-ischemic glucose content after intestinal preservation or on lactate content (data not shown).

Upon reperfusion, a significant rise in vascular perfusion pressure after preservation in UW was observed. Similar phenomena have already been described after preservation of other organs, such as the heart [20] or the liver [17, 23], and they have been attributed to the high potassium content of UW (124 mmol/l), which may have an injurious impact on vascular endothelium, leading to an impaired vascular relaxation after extended preservation periods [6, 20]. Celsior, being an extracellular type preservation solution, contains only small amounts of potassium (15 mmol/l), and this is thought to account for our not finding any major alterations in the vascular perfusion characteristics after storage of the intestines in Celsior. Nevertheless, there were no differences between Celsior and UW with regard to capillary water filtration as judged from the transluminal water loss upon reperfusion. It has been shown that postischemic changes in intestinal vascular permeability are primarily mediated by the impact of oxygen-derived free radicals [10, 19] arising from the xanthine-oxidase reaction upon reintroduction of oxygen into previously anoxic tissue. Both preservation solutions tested in this study are supplemented with antioxidative agents in order to counteract oxygen free radical tissue damage; nevertheless, a significant increase in radical-mediated lipid peroxidation was observed upon reperfusion compared to nonischemic controls. Although it is conceivable that lipid peroxidation would have been even more pronounced if no antioxidant preservative had been used, it can be said that neither UW nor Celsior was sufficiently supplemented to prevent a relevant oxidative tissue insult after 18 h of ischemia in our model. However, Celsior provided better preservation of the small intestine than UW in terms of reduced enzyme leakage upon reperfusion and an increased functional ability of the intestinal mucosa to absorb carbohydrates from the lumen.

We are aware that the interpretation of the present results has to be done cautiously due to the methodological limitations of the experimental in vitro/ex situ model used in this study. In particular, the relatively great potential of the intestinal mucosa to regenerate within several days of reperfusion in vivo [25] could not be accounted for in the reported experimental setting. However, we conclude that Celsior, originally designed for heart transplantation, also seems to be a suitable solution for the preservation of the gut. Subsequent trials, including intestinal transplantation in vivo with long-term follow-up, are strongly encouraged.

References

1. Atkinson DE (1968) The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry 7: 4030
2. Barr ML, Nishanian P, Sakamaki Y, et al (1997) A new organ preservation solution, Celsior, is superior to Euro-Collins and University of Wisconsin solutions in decreasing lung reperfusion injury. Transplant Proc 29: 1357
3. Belzer FO (1993) Evaluation of preservation of the intra-abdominal organs. Transplant Proc 25: 2527
4. Burgmann H, Reckendorfer H, Sperlich M, Spieckermann PG (1992) Small bowel tissue high-energy phosphate alterations during hypothermic storage using different protecting solutions. Eur Surg Res 24: 84
5. DeBruin RWF, Heineman E, Marquet RL (1994) Small bowel transplantation: an overview. Transpl Int 7: 47
6. Delyani JA, Nossuli TO, Scalia R, Thomas G, Garvey DS, Lefer AM (1996) S-nitrosylated tissue-type plasminogen activator protects against myocardial ischemia/reperfusion injury in cats: role of the endothelium. J Pharmacol Exp Ther 279: 1174
7. Dubois RS, Vaughan GD, Roy CC (1968) Isolated rat small intestine with intact circulation. In: Norman JC (ed) Organ perfusion and preservation. Appleton-Century-Crofts, New York, 863
8. Gercken G (1961) Glukose-, Milchsäure-, ATP-, ADP-Konzentrationen im arteriellen und venösen Coronarblut des Hundes bei Sauerstoff-Mangel. Pflügers Arch 272: 323
9. Gonzalez AP, Sepulveda S, Massberg S, Baumeister R, Menger MD (1994) In vivo fluorescence microscopy for the assessment of microvascular reperfusion injury in small bowel transplants in rats. Transplantation 58: 403
10. Granger DN, Höllwarth E, Parks DA (1986) Ischemia-reperfusion injury: role of oxygen derived free radicals. Acta Physiol Scand 548: 47

11. Hirata Y, Taguchi T, Suita S, Takeshige K (1994) Adenine nucleotide metabolism in relation to graft viability in rat small-bowel transplantation. Eur Surg Res 26: 309
12. Isselhard W, Berger M, Denecke H, Witte J, Fischer JH, Molzberger H (1972) Metabolism of canine kidneys in anaerobic ischemia and in aerobic ischemia by persufflation with gaseous oxygen. Pflügers Arch 337: 87
13. Kuroda Y, Sakai T, Suzuki Y, et al (1996) Small bowel preservation using a cavitary two-layer (University of Wisconsin solution/Perfluorochemical) cold storage method. Transplantation 61: 370
14. Menasché P, Termignon JL, Pradier F, et al (1994) Experimental evaluation of Celsior, a new heart preservation solution. Eur J Cardiothorac Surg 8: 207
15. Minor T, Isselhard W (1993) Role of the hepatovasculature in free radical mediated reperfusion damage of the liver. Eur Surg Res 25: 287
16. Minor T, Sturz J, Klauke H, Isselhard W (1995) Reduction of oxidative tissue injury and endothelial dysfunction by graduated reperfusion after cardioplegic arrest in isolated rat hearts. Free Radic Biol Med 18: 621
17. Minor T, Isselhard W, Klauke H (1996) Reduction in nonparenchymal cell injury and vascular endothelial dysfunction after cold preservation of the liver by gaseous oxygen. Transpl Int 9[Suppl 1]: 425
18. Minor T, Klauke H, Isselhard W (1997) Assessment of intestinal integrity after ischemic preservation by luminal and vascular perfusion in vitro. Eur Surg Res 29: 246
19. Parks DA, Granger DN (1983) Ischemia-induced vascular changes: role of xanthine oxidase and hydroxyl radicals. Am J Physiol Gastrointestinal and Liver Physiology 245: G285
20. Pearl JM, Laks H, Drinkwater DC, et al (1994) Loss of endothelium-dependent vasodilatation and nitric oxide release after myocardial protection with university of Wisconsin solution. J Thorac Cardiovasc Surg 107: 257
21. Pisarenko OI, Solomatina ES, Ivanov VE, Studneva IM, Kapelko VI (1985) On the mechanism of enhanced ATP formation in hypoxic myocardium caused by glutamic acid. Basic Res Cardiol 80: 126
22. Pohland CCJ, Toennies SE, Linsel-Nitschke M, et al (1995) Ischemia and reperfusion injury in rat small bowel: evaluation with an ex vivo reperfusion model. Transplant Proc 27: 590
23. Tilser I, Tichy M, Ettlerova E, Holecek M (1996) Effect of supplementation of modified UW solution with OKY-046 on long-lasting hypothermic liver storage of rats. Cryo-Lett 17: 371
24. Toledo Pereyra LH (1979) Small bowel preservation: evolution of methods and ideas, and current concepts. Transplant Proc 24: 1083
25. Wagner R, Gabbert H, Höhn P (1979) Ischemia and post-ischemic regeneration of the small intestinal mucosa. Virchows Arch B Cell Pathol 31: 259
26. Wong SHY, Knight JA, Hopfer SM, Zahria O, Leach CNJ, Sunderman FW (1987) Lipoperoxides in plasma as measured by liquid chromatographic separation of malondialdehyde-thiobarbituric acid adduct. Clin Chem 33: 214
27. Yamada T, Taguchi T, Hirata Y, et al (1994) The effect of hyperbaric oxygenation on ischemia- reperfusion injury of the small intestine. Transplant Proc 26: 1506