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Prolonged rat pancreas preservation using a solution with the combination of histidine and lactobionate

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Abstract. A newly formulated solution consisting of lactobionate with or without histidine was tested in the preservation of the rat pancreas. Adult male Lewis rats weighing 120–250 g were used as donors and recipients. Fifty-four rat pancreas transplants were performed to investigate the effectiveness of this test solution and to compare it with the standard University of Wisconsin (UW) solution. The final osmolarity of the new test solution was 290–320 mosmol/l. This solution had a higher sodium content and lower potassium content (Na: 110 mEq/l, K: 50 mEq/l). Adenosine, insulin, hydroxyethyl starch and dexamethasone, which are components of the UW solution, were not present in this test solution. Histidine was used as a buffer. Rat pancreases were stored at 4 °C in either standard UW solution, or high-Na^+-histidine solution, or high-Na^+-lactobionate solution for 48 h and 72 h prior to heterotopic transplantation into rats with streptozotocin-induced diabetes mellitus. Functional success rates for rats receiving pancreases that had been preserved in high-Na^+-histidine and in high-Na^+-lactobionate solutions at 4 °C were 100% (5/5) and 100% (7/7) after 48 h preservation, and 50% (4/8) and 14% (1/7) after 72 h preservation, respectively. By contrast, standard UW solution gave only a 44% (4/9) success rate after 48 h preservation and a 0% (0/8) success rate after 72 h preservation. These results demonstrated that the high-Na^+-histidine solution was superior to standard UW solution for rat pancreas preservation. This was probably due to the buffer, histidine, which prevented the acidosis of ischemic tissue during the period of preservation.

Key words: Pancreas preservation – Rat – Modified UW solution – Histidine – HL solution

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It has been well established both by experimental models and clinically that University of Wisconsin (UW) solution is a highly effective preservation solution suitable not only for the liver [1, 2], kidney [3] and pancreas [4, 5], but also for the heart [6, 7]. Nevertheless, the mechanism for this remains unclear. Earlier studies by the present authors [8–13] and others [14–16] have demonstrated that not all components of the original UW solution are necessary. The effectiveness of UW solution is still maintained in the study of rat [10, 11, 15, 16], rabbit [8] and human [9] liver preservation, and rat pancreas [13] and kidney preservation [14], if hydroxyethyl starch (HES) and some pharmacological additives are omitted from the solution. We have shown previously that the effectiveness of UW is dependent mainly on the presence of the lactobionate anion and this effectiveness can be maintained or even improved by the use of different sugar moieties or altered cation composition in the preservation of rat liver and pancreas [10, 13].

Our recent experiments have demonstrated the relatively high viscosity of the UW solution due to the presence of HES [8, 11]. It is probable that this feature makes the processing of initial perfusion slow and harmful to the organs because of the outflow block after reflow in rat and dog liver experiments [11, 17, 18]. The relatively limited buffering capacity of the UW solution may also be a disadvantage when preservation time is prolonged. Therefore, a new preservation solution was developed containing lactobionate, but removing those components which are unnecessary or injurious (HES, adenosine, insulin, raffinose) and adding histidine to enhance the buffering capacity. This solution, consisting of histidine and lactobionate, is called HL solution. By using this HL solution we have obtained satisfactory survival in rats receiving liver transplants after 24 h cold (4 °C) storage. In the present study we described our experience in the use of this type of solution for long-term rat pancreas preservation. The effectiveness of this solution in rat pancreas preservation was compared with that of standard UW solution, high-Na^+-UW solution [13] and Eurocollins [EC] solution.

Table 1. Composition of test solutions. (mmol/l). Penicillin and streptomycin were added to both solutions. They were filter-sterilized by a 0.22 μm filter, stored in sealed glass containers, kept in a refrigerator at 4°C and used within 1 week

Solution	high-Na^+-lactobionate solution	high-Na^+-Histidine solution
Na-Lactobionate	110	110
$MaSO_4$	5	5
K-KH_2PO_4	25	25
Raffinose	30	0
Histidine	0	30
Glutathione	3	3
Allopurinol	1	1
Adenosine	5	0
Na^+ (mEq/l)	110 ± 5	110 ± 5
K^+ (mEq/l)	45 ± 5	45 ± 5
Osmolarity (mOsm/l)	315 ± 5	295 ± 5
pH	7.4	7.4

Table 2. The results of 48- and 72-h rat pancreas preservation using Belzer UW solution, Eurocollins solution, high-Na^+-lactobionate solution and high-Na^+-histidine solution. Belzer UW solution (ViaSpan) was from Du Pont Japan and Eurocollins solution, from Green Cross Corporation Japan

Experimental groups	Preservation time	Functional success rate	K value
Belzer UW solution	48 h (n = 9)	44% (4/9)	2.41 ± 0.73
	72 h (n = 8)	0% (0/8)	–
Eurocollins solution	48 h (n = 5)	20% (1/5)	1.16
	72 h (n = 5)	0% (0/5)	
High-Na^+-lactobionate solution	48 h (n = 7)	100% (7/7)	2.23 ± 0.56
	72 h (n = 7)	14% (1/7)	1.65
High-Na^+-histidine solution	48 h (n = 5)	100% (5/5)	2.35 ± 0.74
	72 h (n = 8)	50% (4/8)	2.37 ± 0.53

Materials and methods

Inbred LEW rats (Charles River, Japan) weighing 120–250 g were used as donors and recipients. The methods of total pancreatectomy and heterotopic pancreas transplantation were as we have described previously [13]. Donor segmental pancreas grafts were perfused in situ via the aorta with 2 ml of the test solution containing 20 units of heparin and stored in the test solution at 4°C in a refrigerator. After either 48 h or 72 h of cold preservation, donor pancreases were transplanted heterotopically into the right side of the neck of recipient rats which had been previously rendered diabetic by the administration of streptozotocin (65 mg/kg, i.v.). Graft endocrine function was evaluated by measuring non-fasting blood glucose values on days 1, 3, 5, 7 and 14 post-transplant and performing an intravenous glucose tolerance test (IVGTT) on the 14th post-operative day, following administration of 0.5 g glucose/kg body weight. Normal graft function was defined as random blood glucose values consistently below 200 mg/dl and K values exceeding 1.0 on IVGTT.

The composition of our high-Na^+-lactobionate solution with or without histidine is shown in Table 1. Magnesium sulphate, glutathione and allopurinol were present at concentrations of 20, 3 and 1 mmol/l, respectively, as in the original UW solution. The final osmolarity of both solutions was 290–320 mosmol/l. The pH was adjusted to 7.4 at room temperature.

The buffering capacity was defined as the number of milliequivalents per liter of H^+ required to cause a decrease of 1 pH unit from the initial pH of the solution. This pH change was determined by plotting pH (measured at 15°C) when 1 l of test solution was titrated with 0.1 M HCl. The viscosity of the test solution was measured at 4°C with an Ostwald viscosity meter and expressed as a ratio against distilled ion exchanged water. Statistical analysis was performed using Fisher's test to compare the results of different experimental groups.

Results

A total of 54 rat pancreas transplants were performed and no recipient animals were excluded from the experimental groups. The outcome of these experimental groups is summarized in Table 2. In the 48 h preservation groups, all grafts preserved in high-Na^+-lactobionate solution and high-Na^+-histidine solution showed normal endocrine function (7/7 vs. 5/5) whereas four out of nine grafts preserved in Belzer UW solution recovered normal function and one out of five grafts showed normal function using EC solution. In the 72-h preservation group using the high-Na^+-histidine solution, four out of eight recipients showed satisfactory graft function, while only one out of seven pancreases preserved in high-Na^+-lactobionate solution demonstrated normal graft function. Following 72-h preservation, none of the grafts preserved in Belzer UW solution (0/8) or EC solution (0/5) resulted in normal endocrine function. The differences were statistically significant for the 72-h preservation group, on comparing UW solution with the high-Na^+-histidine solution ($P < 0.05$) but not quite so on comparing the high-Na^+-lactobionate solution with the high-Na^+-histidine solution.

According to our laboratory parameters, the buffering capacities of UW solution, EC solution and high-Na^+-histidine solution were 11.0, 20.0 and 20.4 mEq/l per pH unit, respectively (Fig. 1). The viscosity of each solution, expressed as a ratio compared with deionized water, was 3.32 for the UW solution, 1.21 for the EC solution and 1.20 for the high-Na^+-histidine solution (Fig. 2).

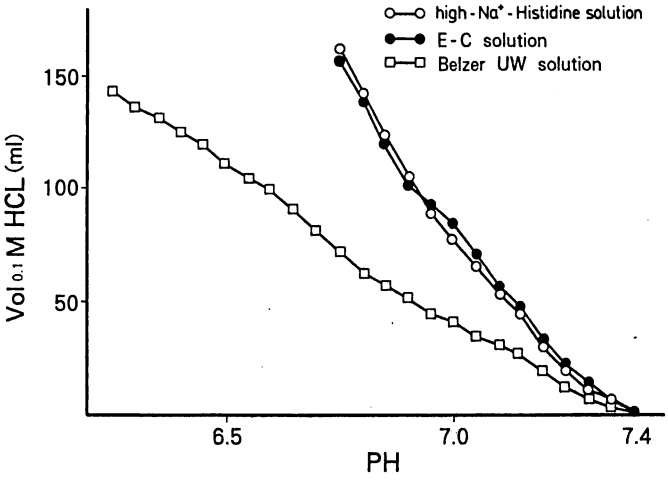

Fig. 1. The buffering capacity of the solutions. One liter of each solution was titrated with 0.1 M HCl, and pH measured at 15°C sequentially and plotted out. High-Na^+-histidine solution represents a substantially greater buffering capacity than does Belzer UW solution. *E-C* = Eurocollins

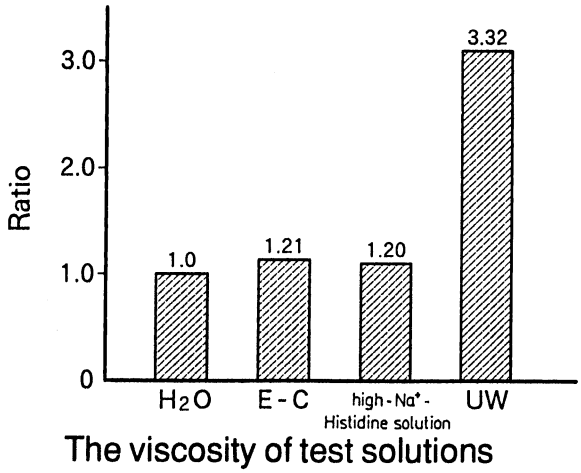

Fig. 2. The viscosity was expressed as a ratio compared with deionized water. EC solution and high-Na^+-histidine solution have similar ratios at 1.21 and 1.20, respectively, while Belzer UW solution has a substantially higher viscosity at 3.32. *E-C* = Eurocollins

Discussion

In our previous report the effectiveness of UW solution was significantly improved by altering the cation composition from high-K^+ to high-Na^+ content in the rat liver preservation study [10]. This was also the case in the rat pancreas preservation study [13]. Using the high-Na^+ solution, similar improved results over standard UW solution have also been reported in dog liver, kidney and pancreas [19] as well as in human liver preservation studies [9]. This improvement with the high-Na^+ solution may be due to the reduction in potassium content leading to decreased vasoconstriction, minimized endothelial cell injury, and ameliorated microcirculation during the initial phase of reperfusion.

It should be pointed out that substitution of the amino acid histidine for raffinose improved survival rates although not to a statistically significant degree. Histidine has pK values of 1.78, 5.97 and 8.97 and appears to be a highly effective buffer in the physiological pH range. A higher concentration of histidine (90 mmol/l) with 20 mmol KH_2PO_4 has previously been shown to result in a potent buffering capacity (buffering capacity: 28 mEq/l per pH unit). But even at the lower concentration of histidine used in the present study, buffering capacity (20.4 mEq/l per pH unit) was adequately maintained and exceeded that of the original UW solution. This was also noted in EC solution. This improved buffering capacity may be the reason for the improved survival when using the high-Na^+-histidine solution.

Raffinose is a trisaccharide and was present in the original UW solution to suppress cell edema during cold storage [20]. However, the relatively low concentration of raffinose (30 mmol/l) in the standard UW solution can be replaced by an equimolar concentration of glucose, without reducing the effectiveness of lactobionate based solutions, in the preservation of rat liver and pancreas [11, 13]. Even a higher concentration of raffinose (190 mmol/l) can be replaced by either equimolar sucrose or glucose in a phosphate buffer based solution in rat liver preservation without loss of effect [21]. The sugar component, irrespective of its molecular weight does not, therefore, appear essential to the preservative capacity of solutions in rat organs. Indeed the present results indicated that a simple sugar moiety may not really be necessary, as far as our new preservation solution is concerned. Replacement of raffinose with histidine cause little difference to the viscosity of the solution (1.22 vs 1.2) and was unlikely to be responsible for the improvement in survival rates noted in this study.

In conclusion, the present results indicated that our new solution used in combination with histidine and lactobionate was more effective than standard UW solution or high-Na^+ UW solution in rat pancreas preservation. The mechanism for this action should be further clarified.

References

1. Kalayoglu M, Sollinger HW, Stratta RJ, et al (1988) Extended preservation of the liver for clinical transplantation. Lancet I:617
2. Todo S, Nery J, Yanaga K, Podesta L, Gordon RD, Starzl TW (1989) Extended preservation of human liver grafts with UW solution. JAMA 261:711
3. Ploeg RJ, Coosens D, Vreugdenhil P, McAnulty JF, Southard JH, Belzer FO (1988) Successful 72-hour cold storage kidney preservation with UW solution. Transplant Proc 20:935
4. Wahlberg JA, Southard JH, Belzer FO (1986) Development of a cold storage solution for pancreas transplantation. Cryobiology 23:477
5. Wahlberg JA, Love R, Landegaard L, et al (1987) Successful 72-hour preservation of the canine pancreas. Transplantation 43:5
6. Makowka L, Zerbe TR, Chapman F, et al (1989) Prolonged rat cardiac preservation with UW lactobionate solution. Transplant Proc 21:1350
7. Wicomb WN, Collins GM (1989) Twenty-four-hour rabbit heart storage with UW solution; effects of low flow perfusion, colloid and shelf storage. Transplantation 48:6
8. Jamieson NV, Lindel S, Sundberg R, Southard JH, Belzer FO (1988) An analysis of the components in UW solution using the isolated perfused rabbit liver. Transplantation 46:512
9. Jamieson NV, Johnston PS, O'Gray JG, et al (1990) Clinical use of UW solution or simplified liver preservation solution prior to transplantation in 179 human livers. Transplant Proc 22:2088
10. Sumimoto R, Jamieson NV, Wake K, Kamada N (1989) 24-hour rat liver preservation using UW solution and some simplified variants. Transplantation 48:1
11. Sumimoto R, Jamieson NV, Kamada N (1990) Examination of the role of the impermeants Lactobionate and Raffinose in a modified UW solution. Transplantation 50:573
12. Sumimoto R, Kamada N, Jamieson NV, et al (1991) A comparison of a new solution combining histidine and lactobionate with UW solution and Eurocollins for rat liver preservation. Transplantation 51:589
13. Urushihara T, Sumimoto R, Jamieson NV, et al (1991) A comparison of some simplified lactobionate preservation solution with standard UW solution and Eurocollins solution for pancreas preservation. Transplantation. In press
14. Biguzas M, Jabloski P, Howden BO, et al (1990) Evaluation of UW solution in rat kidney preservation. II The effect of pharmacological additives. Transplantation 49:1051
15. Marshall VC, Howden B, Jablonski P, Scott DF, Thomas AC, Cham C, Biguzas M, Walls K (1990) Analysis of UW solution in a rat liver transplant model. Transplant Proc 22:503

16. Yu W, Coddington D, Bitter-Suermann H (1990) Rat liver preservation I. The components of UW solution that are essential to its success. Transplantation 49:1060
17. Jamieson NV, Sundberg R, Lindell S, et al (1988) Preservation of the canine liver for 24–48 hours using simple cold storage with UW solution. Transplantation 46:517
18. Jamieson NV (1991) Improved preservation of the liver for transplantation. Alimentary Pharmacol Ther. In press
19. Moen J, Claesson K, Pienaar H, et al (1989) Preservation of dog liver, kidney and pancreas using the Belzer-UW solution with a high sodium and low potassium content. Transplantation 47:940
20. Belzer FO, Southard JH (1988) Principles of solid-organ preservation by cold storage. Transplantation 45:673
21. Kobayashi T, Sumimoto R, Shimada et al (1991) Effective of sugars in the preservation solution on liver storage in rats. Cryobiology 21:1350