Transplant Int (1992) 5 [Suppl 1]: S408–S410

TRANSPLANT
International

A comparison of histadine lactobionate solution with University of Wisconsin solution for rat liver and heart preservation

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Abstract. We developed a new solution mainly composed of Na-lactobionate and histidine (HL) and compared the effectiveness of this solution with that of University of Wisconsin (UW) solution using orthotopic liver and heterotopic heart transplantation in rats. The new solution has a higher sodium content and a lower potassium content (Na, 90 mEq/l; K, 45 mEq/l) than UW. Hydroxyethyl starch, adenosine, dexamethasone and insulin are not included. Buffering capacity is increased by adding histidine (90 mM/l) together with KH_2PO_4 (20 mM/l). Rat liver was perserved in either UW or HL solution hypothermically for 24 h and then transplanted orthotopically into the recipient rat. The heart was preserved in either solution for 18 h and transplanted heterotopically into the recipient rat. The 1-week survival rate for rats receiving livers preserved in UW for 24 h at 4 °C was 29% (5/17). In contrast, the new solution (HL) gave a 78% (11/14) survival rate ($P < 0.01$). The 1-week heart graft survival rate, using UW solution was 50% (3/6), following 18-h cold preservation, whereas all hearts (7/7) continued to beat for over a week using new HL solution ($P < 0.05$). These results demonstrated that the new HL solution, with a substantial buffering capacity, was superior to UW solution in rat liver and heart preservation.

Key words: Rat liver preservation – Rat heart preservation – UW solution – Histidine – Buffering capacity

University of Wisconsin (UW) solution has had a great impact in the field of organ transplantation and preservation [5, 6]. However, clinical heart preservation has not benefited from it, and there still appears to be some room left for its further improvement. We have shown that the UW solution has a high viscosity, a high potassium content, and a low buffering capacity as compared with conventional preservation solutions such as EuroCollins solution, citrate solution and phosphate-buffered sucrose solution. These limiting factors undermine ideal long-term preservation. By ameliorating these points it is, therefore, possible to develop a better preservation solution than standard UW solution. In this experiment we described the use of a new solution composed mainly of Na-lactobionate and histidine (HL) and compared the effect of this solution with standard UW solution employing the orthotopic rat liver and heterotopic heart transplant models.

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Materials and methods

Male Wistar rats for liver transplantation and Lewis rats for heart transplantation were used as donors and recipients respectively.

Rat liver preservation and transplantation. Following the intravenous injection of 200 units of heparin, the donor liver was perfused in situ with 3–5 ml of chilled test solution via the portal vein. The liver was preserved hypothermically (4 °C) in a beaker containing the test solution for 24 h and then transplanted orthotopically into the recipient rat according to the modified techniques originally described by Kamada [7]. Arterial reconstruction was not performed. One-week survival rates were compared.

Rat heart preservation and transplantation. Following the injection of 200 units of heparin, the donor heart was initially perfused in situ with 5 ml of chilled test solution via the suprahepatic vena cava and excised. The heart was perfused with 2 ml of chilled test solution by manual injection via the aortic root and immersed in the test solution at 4 °C. After 18 h of storage, the heart was transplanted in the right side of the neck of the recipient animal according to the modified techniques described by Heron [3]. Graft survival was judged by inspecting and palpating the heart. One-week graft survival rates were compared.

Test solution. The composition of HL solution and standard UW solution is shown in Table 1. The solutions contain penicillin and streptomycin and were filter sterilized and stored at 4 °C and used within 1 week of preparation.

Table 1. Composition of UW and HL solutions (mM/l)

K-lactobionate	100	
Na-lactobionate		90
Histidine		90
$Na\text{-}KH_2PO_4$	25	
$K\text{-}KH_2PO_4$		20
Raffinose	30	25
$MgSO_4$	5	5
Glutathione	3	3
Adenosine	5	
Allopurinol	1	1
Insulin	100 U/l	
Hydroxyethyl starch	5 g%	
Na (mEq/l)	30	90
K (mEq/l)	120	45
Osmolarity	320–330	320–330
pH	7.4	7.4

Table 2. The viscosity of UW, EC and HL solutions. Values are means ($n = 6$ per group)

H_2O	UW solution	EC solution	HL solution
1.0	3.32	1.21	1.33

Table 3. The buffering capacity (mEq/l/per pH unit) of UW, EC,PBS and HL solutions. Values are shown as means ($n = 3$ per group)

UW solution	EC solution	PBS solution	HL solution
11	20	30	28

Viscosity. The viscosity of the test solution was measured at 4 °C using an Ostwald viscosity meter and expressed as a ratio in comparison with the value for deionized water (Table 2).

Buffering capacity. The buffering capacity of the solutions was defined as the number of milliequivalents per liter of H + required to produce a decline of one pH unit from the initial pH of the test solution. This was determined by plotting the pH (measured at 15 °C) when 1 l of test solution was titrated with 0.1 M HCl (Table 3).

Tissue water content. Samples of liver tissue were taken immediately before initial flushing and folowing 24 h and 48 h of cold preservation. The tissue was weighed immediately to give wet weight and then reweighed after being dried overnight in an oven at 105 °C to give the dry weight. The tissue water content could then be calculated from the following formula:

$$\text{tissue water content} = \frac{(\textit{wet weight} - \textit{dry weight})}{\textit{wet weight}} \times 100\,(\%)\ \text{(Table 4)}$$

pH change. The pH change in each test solution was measured at 15 °C before and after 24 and 48 h of liver preservation using a pH meter (Table 5).

Results

The results are shown in Tables 2–6. This new solution has a lower viscosity (1.33 vs. 3.32) and a substantial buffering capacity (30 vs. 10 mEq/l per pH unit) as compared with that of standard UW solution. The results of pH change in the preservation solution surrounding the graft suggested

Table 4. Tissue water content (liver) (%). Values are shown as means with SD ($n = 6$ per group)

	Before flush	After 24 h	After 48 h
UW solution	72.0 ± 0.4	67.8 ± 0.3	67.6 ± 0.7
HL solution	71.6 ± 0.8	67.9 ± 0.5	68.4 ± 0.6

Table 5. The pH values of the preservation solution surrounding the graft. Values are shown as means with SD ($n = 6$ per group)

	Before immersion	After 24 h	After 48 h
UW solution	7.40	7.20 ± 0.03	7.16 ± 0.02
HL solution	7.40	7.38 ± 0.05	7.35 ± 0.04

Table 6. % Graft survival. Rat livers or hearts were preserved for the indicated time and transplanted. Survival was considered 100% if graft was functioning on the 7th day post-transplant. Values given are surviving grafts/total

	UW	HL
Liver 24 h	29% (5/17)	78% (11/14)*
Heart 18 h	50% (3/6)	100% (7/7)**

* $P < 0.05$ vs UW
** $P < 0.05$ vs UW

that the combined histidine-KH_2PO_4 buffer was more effective than the phosphate buffer alone in UW solution.

Hearts preserved in UW solution for 18 h developed a dark red appearance shortly after reperfusion. Three out of six heart grafts failed within 1 week. In contrast, all heart grafts preserved in HL solution retained a normal light pink color after revascularization and effective beating commenced. All seven grafts continued to beat for over a week.

The livers preserved in UW for 24 h developed a mottled appearance on reperfusion, suggesting an area of no reflow and subcapsular hemorrhage. The 1-week survival rate for UW was 29%. Using HL solution, 78% of the rats receiving livers preserved for 24 h survived for over 1 week. In this group, livers rapidly resumed normal color and showed an even perfusion.

Discussion

Since the introduction of UW solution in 1986, preservation times of solid organs have ben markedly extended in animal experiments [5, 14] and in the clinical situation [6, 13]. Nevertheless, it is still unclear whether and to what extent all components contribute to the effect of the UW solution. From our earlier experiments using the rat liver transplant model, we can safely conclude that the dramatic effect of the UW solution results from the use of lactobionate as an anion and the inclusion of fresh glutathione as a radical scavenger [1, 11, 12]. The effect of the other pharmacological components, if any, is masked under the effect of these two components. Moreoever, the apparent limitations of the UW solution are as follows: (1) UW has a high viscosity due to the inclusion of hydroxyethyl starch which does not permit rapid organ perfusion,

(2) the high potassium cation content in the UW solution produces vasoconstriction and endothelial cell injury when used in the initial perfusion [8, 10], and (3) the substantially small buffering capacity of the UW solution augments intracellular acidosis during cold storage that can be deleterious to cell viability if the preservation times are extended.

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. This amino acid can be safely added to the preservation solution without raising the electrolyte content or the osmolarity. Bretschneider's solution [2], which consists primarily of histidine with low concentrations of sodium and potassium, has been widely used experimentally and clinically in the heart, liver [9], and kidney [4]. The HL solution was thus formulated by combining constituents from our previous experiments with lactobionate-based solutions, with histidine as a buffer, to produce a solution containing, what we believed to be, the most important components included in the UW solution and Bretschneider's solution. Although UW solution has been shown to preserve effectively abdominal organs, clinical heart preservation has not benefitted from these advances in preservation, the best results being obtained with a modified UW solution experimentally.

These experiments clearly demonstrated that when using a solution in which Na-lactobionate and histidine were combined it was possible to produce results which were superior to the standard UW solution in rat liver and heart preservation. Although the mechanism of action of histidine has not been fully defined, the results with HL merit investigation and trials in larger animals and in man.

References

1. Boudjema K, Gulik TM, Lindell SL, Vreugdenhil PS, Southard JH, Belzer FO (1990) Effect of oxidized and reduced glutathione in liver preservation. Transplantation 50:948–951
2. Heinemeyer D, Belles G, Stapenhorst K (1987) Intracellular pH measurements during cardiac arrest in ventricular myocardium by Bretschneider's cardioplegic solution HTK and St. Thomas Hospital solution with and without procaine. Thorac Cardiovasc Surg 35:48
3. Heron I (1971) A technique for accessory cervical heart transplantation in rabbits and rats. Acta Pathol Microbiol Scand 79:366
4. Ismer FE, Ludwig A, Schunck O, Bretschneider HJ, Peiper HJ (1988) Kidney procurement with the HTK solution of Bretschneider. Transplant Proc 20:885
5. Jamieson NV, Sunderberg R, Lindell S, Claesson K, Moen J, Vreugdenhil PK, Wight DGD, Southard JH, Belzer FO (1988) Preservation of the canine liver for 24–48 hour using simple cold storage with UW solution. Transplantation 46:517–522
6. Kalayoglu M, Sollinger HW, Stratta RJ, D'alessandro AM, Hoffmann RM, Pirsch JD, Belzer FO (1988) Extended preservation of the liver for clinical transplantation. Lancet 19:617
7. Kamada N, Calne RY (1979) Orthotopic liver transplantation in the rat. Technique using cuff for portal vein anastomosis and biliary drainage. Transplantation 28:47
8. Moen J, Claesson K, Pienaar H, Lindell S, Ploeg RJ, McAnulty JF, Vreugdenhil P, Southard JH, Belzer FO (1989) Preservation of dog liver, kidney and pancreas using the Belzer-UW solution with a high sodium and low-potassium content. Transplantation 47:940–945
9. Pichlmayr R, Bretschneider HJ, Kirchner E (1988) Ex situ Operation an der Leber – eine neue Möglichkeit in der Leberchirurgie. Langenbecks Arch Chir 20:122
10. Sumimoto R, Jamieson NV, Wake K, Kamada N (1989) 24-hour rat liver preservation using UW solution and some simplified variants. Transplantation 48:1–5
11. Sumimoto R, Jamieson NV, Kamada N (1990) Examination of the role of the impermeants lactobionate and raffinose in a modified UW solution. Transplantation 50:573–577
12. Sumimoto R, Jamieson NV, Kobayashi T, Fukuda Y, Dohi K, Kamada N (1991) The need for glutathione and allopurinol in the HL solution for rat liver preservation. Transplantation 52: 565–567
13. Todo S, Nery, Yanaga K, Podesta L, Gordon RD, Starzl TW (1989) Extended preservation of human liver grafts with UW solution. JAMA 261:711–714
14. Wahlberg JA, Love R, Landegaard L, Southard JH, Belzer FO (1987) Successful 72 hour preservation of the canine pancreas. Transplantation 43:5–8