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• *BRIEF REPORTS* •

Evaluation of CMU-1 preservation solutions using an isolated perfused rat liver model

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Abstract

AIM: CMU-1 is a new preservation solution with a low potassium concentration as well as low viscosity that is highly effective in reducing preservation injury. The purpose of this experiment is to compare the protective effect of CMU-1 solution with that of UW during cold preservation and normothermic reperfusion.

METHODS: Wistar rats were divided into two groups according to different preservation solution: CMU-1 group and UW group. After 6, 12 and 24 h cold storage of rat liver in different preservation solutions, the isolated perfused rat liver model was applied to reperfuse the liver for 120 min normothermically (37 ℃) with Krebs-Henseleit solution, meanwhile the pH value of the preservation solution was measured. The perfusate was sampled for the evaluation of alanine aminotransferase (ALT) and lactate dehydrogenase (LDH). At the end of the reperfusion, all of the bile product was collected, energy metabolic substrate and histological examination were performed.

RESULTS: After preserving for 6 h, pH value of both groups did not change; after 12 h, both decreased but with no significant difference. After 24 h, pH value in UW solution group significantly decreased. The total adenine nucleotides level and AEC in liver tissue decreased with preservation time, but they were higher in CMU-1 group. And the amount of bile product after perfusion for 120 min in CMU-1 group was much more than that in UW group. However, there were no significant differences in ALT and LDH levels between two groups. Histology showed no difference.

CONCLUSION: The preservation effect of CMU-1 solution is similar with that of UW solution. However, CMU-1 solution shows some advantages over UW solution in energy metabolism, preventing intracellular acidosis and bile product.

Key words: CMU-1; IPRL; ALT

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INTRODUCTION

For liver transplantation, hypothermic storage with the University of Wisconsin (UW) solution has been regarded as the golden standard[1]. This preservation solution efficiently prevents organ damage and allows prolonged storage of human livers[2], but its high viscosity results in less optimal perfusion and its high potassium concentration requires a pre-flush of liver grafts before reflow in the recipient. The CMU-1, a new preservation solution, was developed by China Medical University for hypothermic graft preservation. In this study, we investigated the preservative effect of CMU-1 solution using an isolated perfused rat liver model.

MATERIALS AND METHODS

Materials

The male Wistar rats (BW 250±20 g, provided by the Animal Center of China Medical University) were divided into groups as following: UW group: After 6 h (T6, $n = 6$), 12 h (T12, $n = 6$), 24 h (T24, $n = 6$) cold storage (40 ℃) in UW solution, the liver was reperfused by Krebs-Henseleit solution (370 ℃) oxygenated with 95% O_2 and 50 mL/L CO_2 for 120 min. CMU-1 group: The treatment was the same as that of the UW group except that the preservation solution was CMU-1 solution instead of the UW solution. The compositions of the UW and CMU-1 solution are listed in Table 1.

Table 1 Composition of UW and CMU-1 solutions

Substrate (mmol/L)	UW	CMU-1
Na^+	30	125
K^+	125	25
$H_2PO_4^-$	25	25
Lactobionate	100	100
Raffinose	30	25
Hydroxyl-ethyl-starch	50 g/L	–
Dextran-40	–	50 g/L
Histidine	–	60
Adenosine	5	–
Allopurinol	1	–
Glutathione	3	–
Insulin	100 IU/L	–
Dexamethasone	8	–
pH	7.4±0.2	7.4±0.2
Osmolarity	320 mosm/L	340 mosm/L
Viscosity	7.5 mpas	5.2 mpas

Methods

The rat livers were procured by Kamamda method and flushed in situ via portal vein by 50 mL 4 ℃ cold storage solution (UW or CMU-1) containing heparin (10 units/100 g) at the height of 40 cm by gravity[3]. The common bile duct was cannulized by a catheter. The liver was preserved in UW or CMU-1 solution for 6, 12 and 24 h. At the end of the preservation, the preservative solution was collected to measure the pH value to evaluate solution buffer capacity. Subsequently, the livers were placed in a perfusion cabinet at 37 ℃, and reperfused with 200 mL recirculating Krebs-Henseleit solution (containing 5% bovine albumin serum and 20 μmol/L sodium taurocholate). The perfusate pressure was 20 cm H_2O and the flow was measured by a monitor as 6-8 mL/min. The perfusate was saturated with O_2:CO_2 (95%:5%)[4,5]. The perfusate samples of 3 mL was collected at 0, 30, 60 and 120 min of reperfusion for determination of ALT and lactate dehydrogenase (LDH) by auto-analysis system.

At the end of each experiment, histological examination was performed in biopsies obtained from the left lateral lobe of livers. For determination of value of ATP, ADP and AMP in liver tissue by high performance liquid chromatography (HPLC), biopsies of 50 mm was taken, chilled in liquid nitrogen and stored at -70 ℃. The value of total adenine nucleotides (TAN) and adenine concentration evaluate (ACE) were calculated as follows: TAN = ATP +ADP+AMP, ACE = (ATP+0.5ADP)/TAN. At the end of perfusion, the bile was collected through the catheter.

Statistical analysis

Results were expressed as mean±SD. The data were analyzed with SPSS10.0; *P* values below 0.05 were considered statistically significant.

RESULTS

Energy material level in liver tissue

With the prolonged preservation time, the level of TAN and AEC decreased. While those of the CMU-1 group reduced more slowly than UW group, and after cold storage, the levels of TAN and ACE in CMU-1 group were significantly higher than those in UW group (Table 2, $P<0.05$).

Table 2 Value of TAN and AEC after perfusate for 120 min

Group	Time (h)	TAN	AEC
UW	6	15.14±2.13	0.56±0.04
	12	14.31±2.10	0.45±0.03
	24	13.44±1.08	0.43±0.02
CMU-1	6	16.15±2.41[a]	0.59±0.03[g]
	12	15.44±1.80[c]	0.51±0.01[i]
	24	14.54±1.89[e]	0.48±0.02[k]

[a]$P<0.05$ CMU-1 *vs* UW group; [c]$P<0.05$ CMU-1 *vs* UW group; [e]$P<0.05$ CMU-1 *vs* UW group; [g]$P<0.05$ CMU-1 *vs* UW group; [i]$P<0.05$ CMU-1 *vs* UW group; [k]$P<0.05$ CMU-1 *vs* UW group.

Bile product after reperfusion

The yellow bile was seen in the common bile duct catheter when the reperfusion began. The result of bile product after 120 min normothermic reperfusion is shown in Table 3. Before 6 h cold storage, no difference was found between the two groups. After 12 and 24 h cold storage, CMU-1 group was significantly higher than that in UW group (Table 3, $P<0.05$).

Table 3 Bile flow after perfusion for 120 min (mL/kg)

Group	6 h	12 h	24 h
UW	244±12	197±3	134±5
CMU-1	249±11	201±4[a]	144±7[c]

[a]$P<0.05$ CMU-1 *vs* UW group; [c]$P<0.05$ CMU-1 *vs* UW group.

ALT and LDH values of the perfusion solution

The ALT and LDH values of CMU-1 group in three preservation intervals were similar to those of UW group. There was no significant difference between the two groups (Tables 4 and 5, $P>0.05$).

Table 4 The value of ALT in the three intervals (IU/L)

Group	0	30	60	120 min
UW				
6 h	17.1±1.2	65.4±2.0	88.7±1.5	321.9±2.7
12 h	28.3±3.5	80.5±1.6	122.1±2.1	408.7±5.6
24 h	52±3.0	108.7±2.5	403.7±3.1	506.7±6.3
CMU-1				
6 h	17.8±2.1	66.1±1.1	87.4±1.8	319.1±5.4
12 h	31.5±1.9	79.9±2.5	125.4±2.9	419.3±6.3
4 h	48.8±1.8	110.3±2.6	398.9±4.1	510.3±4.9

Table 5 Value of LDH in the three intervals (IU/L)

Group	0	30	60	120 min
UW				
6 h	228.5±5.6	378.9±4.7	499.1±10.4	805.2±11.5
12 h	380.2±4.2	480.7±6.7	600.3±11.1	900±13.1
24 h	487.5±3.8	579.6±7.3	1 102±3.5	1 209±12.8
CMU-1				
6 h	218.5±7.4	381.6±5.7	502.4±8.6	811.3±15.6
12 h	407.2±9.1	465.7±3.9	589.7±6.9	904.6±13.9
24 h	478.5±4.3	580.7±2.9	1 109±7.9	1 302±14.2

Histology

After 24 h cold storage, the hepatic lobule structure of the UW group and CMU-1 group was still intact, but some sinusoidal regions became slightly narrow for swelling hepatocytes. The hepatic cords were still clear. The sinusoidal endothelium were normal with mild swelling and rounding. Histology showed no significant difference between the two groups.

The pH value of preservation solution

After 6 h cold storage there was little change in the pH values of the two solutions. After 12 h, the values of UW and CMU-1 solution was reduced to 7.32±0.03 and 7.35±0.02, respectively, there were no significant differences between the two groups. While after 24 h, the value of UW solution

was 7.0±0.03, but the value of CMU-1 solution remained 7.3±0.02. There was a significant difference between the two groups after being preserved for 24 h.

DISCUSSION

Though UW solution is considered as the standard preservation solution for liver, it still has some shortcomings such as high potassium, high viscosity, poor buffering capacity and so on. CMU-1 solution developed by China Medical University is a kind of hypernatrium solution. The main features are the following: (1) CMU-1 solution is a high-sodium, low-potassium solution that can avoid endothelial injury of the blood vessel during reperfusion. Liver preservation in high sodium solution would reduce damage to sinusoidal endothelial cells and hepatocytes[6]. It was hypothesized that Kupffer cells should be less activated and reperfusion injury reduced with the rat liver in cold storage of high-sodium solution[7-9]. (2) It has a potent buffer system-histidine along with the phosphate, which is effective in preventing the tissue from acidosis. Histidine as an impermeable anion can effectively prevent cells from swelling. (3) The colloid in CMU-1 solution is Dextran-40 instead of HES. The viscosity of the solution reduced from 7.5 to 5.2 mPas. Dextran-40 can prevent red cells congregate, protect endothelial cells and improve the liver microcirculation during preservation and reperfusion[10].

Under ischemic preservation conditions, production of ATP is reduced[11]. Depletion of ATP sources can result in disruption of cell homeostasis, as evidenced by the inability to keep the Na^+-K^+ pump, consequently, water moves intracellularly due to the high osmotic gradient resulting in cell swelling. In this condition, the extracelluar osmotic and electric charge gradients formed mainly by the impermeable anion (histidine and raffinose) and colloid (Dextran-40) in the preservation solution can effectively prevent water move intracellularly[12]. Furthermore, in hypothermic preservation condition, the Na^+-K^+ATPase on the hepatocelluar membrane is still active, and its activity is related to the intracellular concentration of Na^+ and extracellular concentration of K^+[13]. In the hypernatrium solution, the active Na^+-K^+ATPase can keep the balance of the ion and cellular activity. High potassium not only damages the blood vessel, but also inhibits the activity of the Na^+-K^+ATPase and delay the graft recovery after reperfusion[14]. Therefore, the hypernatrium solution can protect the hepatocytes and sinusoid endothelial cells effectively, while avoiding high potassium injury.

The ATP level in liver tissue is in direct proportion to the survival rate after transplantation[15]. In this study, the levels of TAN and ACE in CMU-1 group were significantly higher than those of the UW group after preservation, as a result of the bile flow, which is the sensitive index of liver function. These results may be related to the histidine, which can act as the metabolic substrate during the preservation[16].

As a neutral amino acid, the isoelectric point of histidine is 7.59, which is similar to the pH value of the preservation solution, so it acts as a potent buffer system to prevent acidosis[17,18]. The results of pH value in CMU-1 and UW solutions after 24 h cold storage demonstrated that the CMU-1 solution has potent buffer capacity and can effectively prevent intracellular acidosis.

Common parameters in the assessment of I/R injury in the liver are the release of ALT and LDH into the blood or perfusate. These enzymes are released only after the cell membrane has been injured, and the late phenomenon of I/R injury which indicates the damage of hepatocytes. In this study we found that there was no obvious difference between CMU-1 and UW groups in the three intervals. The results of ALT and LDH showed that the damage to the hepatocytes was similar, and CMU-1 and UW solution had the same liver protection effect after 24-h cold storage, as indicated by histology.

Bile production during reperfusion is a commonly applied marker of liver function and is determined by microcirculatory perfusion and the quality of hepatocytes[19]. It is reported that intracellular ATP level is highly correlated to bile production in the early reperfusion phase after transplantation of hypothermically stored rat livers[20]. In this study, no differences in bile production during 120-min reperfusion could be demonstrated between the differently preserved groups before 6-h cold storage, but after 12-h cold storage, the difference between CMU-1 group and UW group appeared. Especially after 24-h cold storage, the difference became more obvious. This result was correlated with the changes of TAN and ACE.

From the above results, we concluded that CMU-1 and UW solutions have the same rat liver protective effect. In the view of liver energy metabolism, preventing intracellular acidosis and bile production, the CMU-1 solution is superior to UW solution.

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