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Comparison of solutions for preservation of the rabbit liver as tested by isolated perfusion

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Abstract The University of Wisconsin (UW) solution consists of a relatively complex mixture of agents. In this study we compared simpler preservation solutions, namely, histidine-tryptophan-ketoglutarate (HTK) and phosphate-buffered sucrose (PBS) with different compositions of UW solution in the isolated perfused rabbit liver model. Livers were stored cold for 24 and 48 h. After 24 h of preservation, the amount of bile produced in UW-preserved livers was significantly greater ($P < 0.05$) than that in HTK-preserved livers. Also, there was less LDH released into the perfusate in UW-preserved livers. There was more edema and lower K +/Na + rations in HTK-preserved livers than in UW-preserved livers (all data $P < 0.05$). After 48 h of preservation, the differences between livers preserved in UW or HTK solution were less noticeable than at 24 h and bile production was similar. LDH and AST release were greater in HTK-preserved livers than in UW livers, but these differences were not statistically significant. Preservation in PBS for 48 h was worse than in either UW or HTK solution. Substitution of polyethylene glycol (PEG) for hydroxyethyl starch (HES) in 48-h UW-preserved livers was not effective. We conclude that solutions simpler in composition than UW solution may be effective in kidney transplantation but do not appear suitable for successful liver preservation.

Key words Liver preservation, rabbit · Preservation, liver, rabbit · UW solution, HTK solution, liver preservation · HTK solution, UW solution, liver preservation

Introduction

The University of Wisconsin (UW) organ preservation solution has been shown to effectively preserve the dog liver for 48 h [12]. However, clinically, it has been recommended that livers should be transplanted within about 24 h since longer periods of preservation lead to a greater incidence of primary nonfunction [5]. It is unclear whether this is due to factors unrelated to preservation, such as donor or recipient factors. However, improvements in preservation solutions could lead to better long-term preservation of human livers.

Recently, other preservation solutions have been shown to be relatively effective for kidney and heart preservation, and these may also be effective for the liver. Histidine-tryptophan-ketoglutarate (HTK), developed as a cardioplegic solution, contains a large concentration of histidine and is thought to be an effective organ preservation solution because of its high capacity to buffer the pH [14]. Phosphate-buffered sucrose (PBS) has been found to be effective for kidney preservation [16]. It is a simple solution containing phosphate and a large concentration of sucrose, as an impermeant. Also, UW solution has been modified to contain polyethylene glycol (PEG) in place of the colloid, hy-

Table 1 Bile production, lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) release during cold storage of the rabbit liver. Values given represent means ± SEM

Preservation solution	*N*	Preservation time (hours)	Bile (ml/2 h per 100 g)	LDH (units/100 g)	AST (units/100 g)
UW	6	0	12.6 ± 1.3	154 ± 20	31 ± 4
UW	9	24	8.4 ± 0.5	375 ± 38	63 ± 7
HTK	5	24	6.5 ± 0.8*	792 ± 207*	96 ± 32
UW	9	48	4.6 ± 1.5	881 ± 136	117 ± 17
HTK	5	48	4.3 ± 1.1	1494 ± 460**	158 ± 21
PBS	3	48	2.7 ± 1.4	2776 ± 170*	173 ± 55
UW, PEG-8	4	48	3.7 ± 1.8	1523 ± 258*	174 ± 40
UW, PEG-20	5	48	1.8 ± 0.7	2134 ± 522*	244 ± 45*

* $P < 0.05$ vs UW solution;
** $P = 0.06$ (marginally significant vs UW solution)

droxyethyl starch, and appears to be good at preserving the rabbit heart [29].

A direct comparison of the effectiveness of these solutions with UW solution for liver preservation has not previously been done. In the present study we have done this, using the isolated perfused rabbit liver preserved for 48 h. We chose 48 h of preservation since, after 24 h in UW solution, there is only minimal injury to the liver, whereas 48 h induces a reproducible and relatively large degree of injury to the liver, including the release of intracellular enzymes and a reduction in bile production [13]. Previous studies have suggested that the isolated perfused liver model is reliable and easily applicable [6]; bile production appears to be a good measure of liver viability [15] and reflects integrated metabolic capabilities of the liver, including intact cellular membranes and effective energy production and utilization. The preservation solutions compared include PBS, HTK, UW solution, and UW solution with PEG in place of hydroxyethyl starch.

Materials and methods

Livers were obtained from New Zealand white rabbits of mixed gender weighing an average of 1.5–2.0 kg. The methods of organ procurement, vascular flushout, preservation (4 °C), and isolated reperfusion have been described previously [11, 13]. The liver was flushed out in situ with cold (4 °C) preservation solution delivered from a height of 50 cm. Flushout was achieved with 250–350 ml of preservation solution according to the respective manufacturer's protocols. For optimal organ procurement with HTK (250–300 ml/kg body weight) 350 ml was always used in our small rabbit livers for adequate equilibration. After flushout and cooling, the liver was excised, the gallbladder removed, and the cystic duct ligated. The liver was then placed in cold storage solution and stored at 4 °C for 24 or 48 h.

Prior to attachment to the isolated perfusion apparatus, the liver was flushed out with 100 ml Ringer's lactate (room temperature, about 20 °–22 °C). It was reperfused through the portal vein at a flow rate of 3 ml/min per gram. The perfusate was Krebs-Henseleit bicarbonate (KHB) containing 4 g % bovine serum albumin for oncotic pressure (fraction V, Sigma Chemical, St. Louis, Mo., USA) and 50 mg of both streptomycin sulfate and penicillin G (Sigma). The perfusate was recirculated (about 1000 ml) and continually oxygenated with O_2:CO_2 (95 %:5 %). The perfusate temperature was maintained at 37 °C.

Bile was collected at 15-min intervals and combined to give total bile produced per 2 h. Perfusate samples were collected at the end of reperfusion (2 h) and used for measurement of lactate dehydrogenase (LDH) and aspartate aminotransferase (AST). The chemicals were purchased from Sigma Chemical. The methods used have already been fully described elsewhere [11, 13]. The preservation solutions were prepared as described by Wahlberg et al. (UW solution) [27], Lam et al. (PBS) [16], and Bretschneider et al. (HTK) [1]. Results shown represent the means and standard error of the means. Means were statistically compared with Student's *t*-test (InStat, Graph Pad, San Diego, Calif., USA). Results are expressed per 100 g liver tissue.

At the end of 2 h of perfusion, five small pieces of each liver in each group were weighed wet, blotted gently on filter paper, and dried in an oven for determination of total tissue water (TTW). After dry weight, these pieces were digested in HNO^3 for determination of K^+ and Na^+ by flame photometry. Results are also expressed as means and standard error of the means (Student's *t*-test).

Results

Livers stored cold in UW solution showed a reduction in bile production from 12.6 ± 1.3 ml/2 h per 100 g to 8.4 ± 0.5 ml/2 h per 100 g (Table 1). There was a greater reduction in livers stored cold in HTK (6.5 ± 0.8 ml/2 h per 100 g) for 24 h, and the differences between UW- and HTK-preserved livers were significant. The amount of LDH released into the perfusate increased from 154 ± 24 units/100 g to 375 ± 38 units/100 g in livers stored in UW solution for 24 h and was significantly higher in livers stored in HTK for 24 h (792 ± 207 units/100 g). For unknown reasons, there was a wide variance in the HTK group fo LDH release (24- and 48-h cold storage). AST was also higher in livers stored in HTK than in UW solution for 24 h, but the differences were not significant.

Cold storage of rabbit livers for 48 h in UW or HTK solution had a similar effect on bile production, which was reduced by about 65 % from the control (0-h preservation) value of 12.6 ± 1.3 ml/2 h per 100 g to about 4.6 ± 1.5 ml/2 h per 100 g (Table 1). Preservation in HTK solution, however, resulted in a greater degree of hepatocellular injury, as indicated by the greater release of enzymes into the perfusate. LDH was signifi-

Table 2 Total tissue water (*TTW*) and K^+/Na^+ ratio during cold storage of the rabbit liver. Values given represent means ± SEM

*1 P + *2 $P < 0.05$ for HTK vs UW (24 h of preservation); *3 P + *4 $P < 0.05$ for HTK + PBS + PEG 20000 vs UW (48 h of preservation)

Preservation solution	*N*	Preservation time (hours)	TTW kg/kg dry weight	K^+/Na^+ ratio
UW	6	0	2.29 ± 0.12	11.0 ± 1.1
UW	9	24	2.59 ± 0.09	7.7 ± 0.8
HTK	5	24	3.06 ± 0.12*1	4.7 ± 0.6*2
UW	9	48	2.55 ± 0.16	6.5 ± 0.9
HTK	5	48	3.34 ± 0.34*3	4.6 ± 0.5*4
PBS	3	48	3.28 ± 0.25*3	2.8 ± 1.0*4
PEG-8000	4	48	2.49 ± 0.09	5.5 ± 0.5
PEG-20000	5	48	2.92 ± 0.12*3	3.1 ± 0.8*4

cantly lower (881 ± 136 units/100 g) in UW livers than in HTK livers (1494 ± 460 units/100 g) after 48 h of preservation. AST was also higher in HTK-preserved livers than in UW livers, although the differences were not significant.

Cold storage in PBS resulted in greater damage to the liver at 48 h of preservation (Table 1) than in UW or HTK. Bile production was reduced to 2.7 ± 1.4 ml/2 h per 100 g (not significantly different from UW or HTK) and there was a relatively large release of LDH and AST during reperfusion compared to UW or HTK.

Two groups of livers were preserved for 48 h with the UW solution in which hydroxyethyl starch was omitted and replaced by either PEG-8000 (PEG-8) or PEG-20,000 (PEG-20). With both types of PEG bile production was lower than with UW- or HTK-preserved livers, although the differences were not significant. However, there was a significantly greater increase in the amount of LDH released from livers preserved in PEG-8 and PEG-20 than from those preserved in UW solution, as well as in the amount of AST released from livers preserved in PEG-20 versus those preserved in UW solution.

All groups were tested for their capacity to maintain their membrane stability and, consequently, to prevent cell swelling. With increasing preservation time, UW solution shows no differences in TTW content and only a slight reduction in the K^+/Na^+ ratio (Table 2). However, in both the 24-h and 48-h preservation models, HTK, PBS, and PEG-20 showed progressive membrane permeability and membrane instability, as reflected by increasing TTW content and decreasing K^+/Na^+ ratios compared to UW solution (Table 2).

Discussion

An understanding of which components of a preservation solution contribute to improved liver preservation would certainly help in developing optimal liver preservation solutions. To study the effects of each component of a preservation solution on liver function, one would have to conduct a considerable number of experiments. The cost and effort involved would be great while the results might only be equivocal, unless each component were to be tested in various combinations. Another approach would be to compare different preservation solutions that utilize different components to achieve optimal liver preservation. Differences in preservation quality might suggest that a component of one solution is either effective or injurious in long-term liver preservation. This method, which was used in the present study, could reduce the number of experiments that are needed and make it possible to quickly determine which agents are most effective in long-term liver preservation.

In this study we compared three different liver preservation solutions to UW solution. HTK has been found to be effective for liver preservation [7, 8, 28] as it contains a large concentration of histidine, which is a relatively good hydrogen ion buffer at the pH used for liver storage. It has been suggested that the beneficial effects of HTK in kidney, heart, and liver preservation are due to the increased hydrogen ion-buffering capacity of this solution. Recently, Sumimoto et al. [24] have shown that the addition of histidine to UW solution improved rat liver preservation as tested in the orthotopic transplant model. However, a further study by Sumimoto et al. [25] showed that an amino acid (isoleucine) with relatively poor hydrogen ion buffering at a neutral pH could effectively replace histidine. This suggests that the beneficial effects of histidine are not due to its hydrogen ion-buffering capacity but possibly to its relative impermeability across the plasma membrane. Thus, histidine may act like lactobionate in UW solution, as an impermeant for suppression of hypothermic-induced cell swelling.

In this study, HTK solution was not as effective for liver preservation at 24 h as UW solution; it induced a larger decrease in bile production and a greater release of intracellular enzymes from the liver. This suggests that livers preserved in HTK had greater metabolic and structural damage than those preserved in UW solution. Also, in a recent prospective, randomized clinical trial comparing UW and HTK solution in liver transplantation, hepatocellular injury (SGOT, SGPT, LDH, lactase) appeared to be more marked in the HTK group. However, overall evaluation criteria were not

significantly different in the two groups [4]. After 48 h of preservation, the quality of the HTK- and UW-preserved livers, as tested by isolated normothermic perfusion, was similar.

PBS is a simple preservation solution containing a hydrogen ion buffer (phosphate) and a large concentration of an impermeant, sucrose. PBS has been found to be effective for kidney preservation in dogs and humans by Lam et al. [16, 17]; however, Lindell et al. [18] found PBS to be less effective than UW solution. In liver preservation, PBS was not as effective as HTK or UW solution, as indicated by the greater release of intracellular enzymes after 48 h of preservation and 2 h of normothermic reperfusion. The differences in preservation quality between the solution containing sucrose (PBS) and UW solution may be due to the sucrose, which Jamieson et al. [11] have shown cannot effectively replace raffinose in UW solution.

Polyethylene glycol (PEG) has been shown to have properties that may make it suitable for cold storage of organs, [26] including its ability to suppress cell swelling [23] and antioxidant properties [19]. A solution similar to UW, containing PEG in place of hydroxyethyl starch, has been found suitable for rabbit heart preservation [30]. Furthermore, it has been suggested that preservation of organs (heart and liver) in a solution containing PEG can reduce rejection [7, 19, 21]. In the present study, the replacement of hydroxyethyl starch with PEG-8 was less effective for 48 h of liver preservation than UW solution, while PEG-20 caused a greater release of liver enzymes into the reperfusion medium than either HTK or UW solution. Thus, PEG appears to be toxic to preserved livers. This may be due to the source of PEG. It may be necessary to treat PEG by filtration through specific filters, such as those used by Wicomb et al. [29, 30], in order to remove potentially toxic materials.

Because of the poor 48-h preservation PEG data, no 24-h preservation experiments were carried out with this solution.

Since one of the principles of organ preservation is the prevention of cell swelling, we also looked at this, as reflected in TTW content and, consequently, membrane permeability. There was no change in TTW content with increasing cold storage time in UW solution. Tissue that retains K^+ and extrudes Na^+ is capable, by a high K^+/Na^+ ratio, of efficiently carrying out integrated metabolic reactions. Although these values decrease during cold storage, UW-preserved livers had significantly higher K^+/Na^+ ratios and a lower TTW content than the other solutions during 24 h and 48 h of preservation.

The goal of this study was to determine whether HTK, PBS, or PEG could preserve the liver as well as or better than UW solution. Only by comparing different solutions can we determine which components are beneficial to liver preservation, and from these data improved liver preservation solutions can be developed. Currently, it is felt that adequate liver preservation is only achieved (clinically) for about 24 h. It is clear, however, that in the laboratory (dog model), adequate liver preservation can be obtained for 48 h with UW solution [3, 12]. The difference is that, in the laboratory, the donor and recipient are healthy, and under these conditions UW solution is very effective. In clinical transplantation, however, the quality of the donor liver is often questionable (due to age, hypotension, use of different medications, nutritional factors, etc.) and the recipient is, of course, ill. These factors have a great impact on the outcome of liver transplantation. The limit, therefore, is not so much related to poor preservation as it is to donor and recipient factors. Thus, improving preservation solutions for cold static storage may be difficult to show conclusively, either in the laboratory or in the clinic. It may just be that we have reached the limits of cold static storage of the liver (i.e., 48 h).

Placing a liver (or any organ) in the cold without continuous perfusion slows down the rate, but does not stop the inevitable process, of cell and tissue death. What causes cell death is not clear but is probably related to the loss of energy (ATP) and the myriad events that are the sequelae of this, including cytoskeletal structural changes, vascular injury, loss of metabolites, etc. Thus, cold static storage of organs may have a limit of 2–3 days; after this time the organs are not ideally preserved. A better way of accomplishing our goal of excellent quality preservation for 24–48 h may be through continuous machine perfusion, a method that appears effective and possibly superior to cold storage of the kidney [20] and the liver [9, 22].

In conclusion, none of the preservation solutions tested in this study were superior to UW solution for preservation of the rabbit liver. Our intention, however, was not to demonstrate superiority of one solution over another, but rather to determine the relative effectiveness of the different solutions. Certainly, if we had observed better preservation with HTK or PEG than with UW solution we would want to pursue the matter further, to seek reasons for these differences and to try to combine, for instance, histidine with UW, or modifications of PEG with UW, to enhance liver preservation. However, this was not the case, and we can only conclude that UW solution continues to be superior in this model.

The lack of clear statistical significance was not our goal; rather we wanted to see how these different solutions compared in a classical reperfusion liver model. What makes UW solution so effective is not entirely clear, although lactobionate appears to have unique properties that make it very suitable for organ preservation. Lactobionate is an effective impermeant and suppresses cell swelling [21]. None of the tested solutions

with ingredients assuming to mimic this function were successful. Lactobionate also chelates calcium [2] and this may be important in preventing calcium activation of hydrolytic enzymes. Finally, lactobionate has been shown to chelate iron and reduce iron-induced generation of oxygen-free radicals [10].

Improvements in liver preservation still need to be mode since a greater incidence of primary nonfunction has been reported in livers preserved for more than 20 h in UW solution. Comparisons between different preservation solutions, while unable to provide information about the mechanisms of preservation effectiveness or injury, make it possible to quickly determine which components of a solution may be suitable for further study.

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