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Comparison of histidine-tryptophan-ketoglutarate (HTK) solution versus University of Wisconsin (UW) solution for organ preservation in human liver transplantation

A prospective, randomized study

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Abstract Over a 30-month period, 60 patients (30 in each group) suffering from end-stage liver disease or primary hepatic malignancy and scheduled for liver transplantation were enrolled in a prospective, randomized study to compare two methods of liver preservation: histidine-tryptophan-ketoglutarate (HTK) solution versus University of Wisconsin (UW) solution. Entry criteria for both groups were: age (18–65 years), elective surgery (transplantable or urgent category of the recipients), first transplantations and harvesting procedure performed by the same team. The parameters under investigation were the clinical and laboratory data pre- and post-transplantation, as well as follow-up data such as complications and survival. There were no significant differences in the two groups as far as the evaluation criteria were concerned, even when cold ischemia time was more than 15 h ($n = 7$). A slight, yet not significant, increase in late complications of the biliary anastomoses could be seen in the UW group. Hepatocellular injury (SGOT, SGPT, GLDH, lactate) appeared to be more marked in the HTK group. These results suggest that both HTK and UW solutions are appropriate for clinical use in liver transplantation, even if cold ischemia time is more than 15 h.

Key words HTK solution
UW solution, liver preservation
Liver transplantation

Introduction

The most commonly used solution for organ preservation in human liver transplantation is University of Wisconsin (UW) solution. In recent years some European centers have introduced Bretschneider's histidine-tryptophan-ketoglutarate (HTK) solution for experimental and clinical liver transplantation [6, 9, 10]. Jamieson et al. [11] were able to show in a canine model that organ survival was possible with a cold ischemia time of up to 48 h when using UW solution. These results, however, could not be reproduced by our group in a minipig model. However, we were able to demonstrate reproducible survival rates of 80% up to 10 h of cold ischemia with HTK solution [5], results which compare well with those of other groups using a similar pig model [14]. Encouraged by the results from animal studies and a clinical pilot study with HTK solution [6], we embarked on a prospective, randomized study at our center to compare the standard procedure with UW solution for organ procurement during orthotopic liver transplantation with that of HTK solution.

Previous publication about HTK solution for human liver preservation have only focused on case-control observations or experiences in a small, uncontrolled series [8–10]. The study presented here is, thus far, the first prospectively randomized one comparing both solutions for human liver preservation. The hypothesis to be proved here was the equivalence of both solutions with regard to initial nonfunctioning and 1-year graft survival. The reason for focusing on HTK solution was to proceed with only one solution for the preservation of all organs.

Patients and methods

Over a 30-months period, 60 patients were enrolled in the study. They were divided into two groups of 30 patients each, and they were followed up for at least 3 months. To obtain comparable groups of patients, the entry criteria for the study were as follows:

1. Age 18–65 years
2. Diagnosis end-stage liver disease or primary hepatocellular malignancy
3. First transplantation
4. Transplantable or urgent stage
5. Harvesting operation performed by our own team.

The evaluation parameters were the following:

1. Need for fresh-frozen plasma during the operation and the ICU stay
2. Length of stay in ICU
3. Laboratory data for the first 3 postoperative days (SGOT, SGPT, GLDH, fibrinogen, TPZ, lactate)
4. Rejection episodes during the 1st month
5. Severe infection during the 1st month
6. Complications, particularly with regard to the biliary anastomosis
7. Survival time and/or cause of death (if applicable).

All harvesting procedures were performed in a standardized way [7] by our team (by only two surgeons: J. E. and R. L.). The solution was chosen in a random fashion after an organ had been offered and accepted and the recipient was categorized as transplantable. If, upon arrival of the surgical team, an organ was judged as unacceptable or for other organizational reasons had to be rejected (solution not accepted by other teams, e.g., for pancreas procurement), the organ was withdrawn and not used for the study but transplanted and registered (30% of the procedures).

In the HTK procurement group, 300 ml cold solution/kg body weight was perfused in equal amounts via the aorta and the portal vein (i.e., 20 l for a 70-kg donor). In the UW procurement group, a total of 4 l was perfused (2 l via the aorta and 2 l via the portal vein). The abdominal cavity was cooled with iced Ringer's solution during a perfusion period lasting 10 min. Special attention was paid to thorough flushing of the biliary duct. In both groups the solutions were infused with the aortic line about 150–200 cm and the portal one about 100 cm above patient level. The excised organ was then packed in bags with melting ice and submerged in the respective solution for transport. During the back bench procedure, the HTK livers were flushed with 500 ml of cold HTK solution through the portal catheter. This line was used for another 500-ml flush during warm ischemia. The livers preserved with UW solution were flushed with 200–300 ml UW solution during the back bench procedure. Some 500–700 ml of cold Ringer's and albumin solution (5%) were injected through the portal line during warm ischemia [1].

The organs were assessed during the harvesting procedure as described recently by Gubernatis et al. [8]. A frozen section was not routinely performed. Organs assessed as stage 4 were not accepted for the patients in this study (Table 1).

A pump-driven portofemoro-subclavian bypass was performed in all recipients. In both groups the anastomoses were opened in the following way: infrahepatic vena cava, hepatic artery, suprahepatic vena cava, and vena porta. The gallbladder was left in situ whenever possible ($n = 21$ in the HTK group and n 24 in the UW group). Two red rubber T-tubes were inserted in the choledochus anastomosis and the gallbladder, respectively. Eight weeks after transplantation the drains were removed.

Table 1 Donor and recipient characteristics. Values given are means. Standard deviations are indicated within parentheses

Evaluation criteria	HTK group	UW group	*P* value
Number of patients	30	30	
Donor age	37.5 (12.6)	31.4 (12.6)	0.0433
Stay in ICU (donors, days)	5.2 (2.1)	5.4 (2.5)	0.467
Quality of the graft (1–4)[a]	1 (12) 2 (13) 3 (5) 4 (–)	1 (15) 2 (12) 3 (3) 4 (–)	0.261 0.293 0.417
Local/external harvesting	17/13	11/19	
Gold ischemia time (min)	579.6 (189.8)	563.6 (132.7)	0.532
Warm ischemia time (min)	68	73	0.294
Recipient age (years) distribution	43.5 (11.8)	41.4 (13)	0.309
Initial non-functioning grafts	1	2 (1)[b]	
Graft survival 3rd month	87%	80%	0.213
Actuarial patient survival at 30 months	77%	74%	0.347
Recipient diagnosis: Cholestatic disease	6	7	
Parenchymatous disease	21	20	
Malignancy	3	3	
Urgency code (ET): Transplantable	16	12	0.204
Urgent	14	18	0.238

[a] Assessment according to the Hannover group proposal [8], with 1 as best and 4 as worst
[b] One liver showed delayed function

For immunosuppressive therapy, an initial dose of 100 mg hydrocortisone was given intraoperatively; thereafter, 2 mg/kg imuran was added, as well as 1 mg/kg cyclosporin, adapted to the plasma level, which was monitored at daily intervals. A 50-mg dose of prednisone was commenced on the 1st day and was tapered to 10 mg/day on the 5th postoperative day.

Liver biopsies were routinely taken between days 5 and 8, or otherwise if clinically indicated. Antibiotics were prophylactically administered (4 g cefotaxim, 10 g azlocillin) for 5 days. All patients were treated by selective decontamination of the digestive tract (SDD).

Statistical analysis was performed using Student's t-test for clinical donor and recipient data and an ANOVA for all laboratory parameters. P-values less than 0.05 were considered significant. The research hypothesis was to prove both solutions to be equivalent.

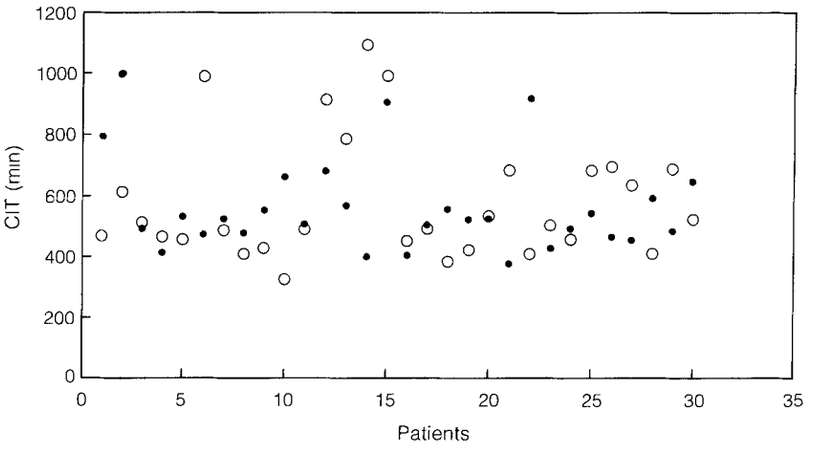

Fig. 1 Comparison of the cold ischemia time *(CIT)* in the HTK group (○) and in the UW group (•). Seven patients received grafts in which CIT exceeded 15 h (four in the HTK group and three in the UW group)

Results

The mean age of the donors was 37.5 years (12.6 SD) for the group treated with HTK solution versus 31.4 years (12.6 SD) for the group treated with UW solution; this difference was significant but should not be overemphasized in the comparison of the absolute donor age. There was no other statistically significant difference in the donor and recipient data or in the indications for transplantation in the two groups (Table 1).

Five livers exhibited impaired behavior (e.g., patchy surface or edema formation) during perfusion ($n = 3$ in the UW group and $n = 2$ in the HTK group) and were subsequently withdrawn from their respective series.

In the HTK group, one liver did not function initially. The patient was retransplanted 2 days later as an emergency and died 10 days later of septic complications. During the 3-months observation period, another three patients died: one after prolonged pneumonia showing excellent liver function, one of myelodystrophy of the brain stem with intact liver function, and third of hepatic artery thrombosis with septic complications.

During the 30-months period, another three patients died: one of recurring hepatocellular carcinoma, one of fulminant recurrence of hepatitis B, and the third of septic shock caused by recurring obstructive episodes of the biliary anastomosis.

One patient was reoperated because of a progressive stenosis of the choledochus anastomosis. A reconstruction was performed using a conduit of the gallbladder. The patient is now well, 10 months after the revision.

The overall survival of the 30 patients in the HTK group over the 30-months period was 77%.

In the UW-series, one liver did not function initially. The patient was retransplanted twice and died 9 weeks after the third transplantation of septic shock. Another patient suffering from primary biliary cirrhosis (PBC) died 3 weeks after the successful tranplantation of an acute, intracerebral bleed. During the 3-month observation period, another three patients died in this group, two of recurrent tumor growth with normal liver function and one of a biopsy-proven fulminant rejection episode.

During the 30-months period, another three patients died. One developed myelodystrophy of the brain stem cause unknown and two patients died of biliary stenosis and septic complications. An adult female patient is still suffering from a stricture of the biliary anastomosis (donor 4 years of age, recipient with primary biliary cirrhosis, 27 months after transplantation). She has not yet been reoperated.

One patient is now, 24 months postoperatively, suffering from chronic rejection. He was switched to FK 506 treatment and has since recovered normal liver function.

In neither series did we find any correlation between prolonged ischemia time and ensuing complications or death. The minimum cold ischemia time was 384 and 378 min, the maximum 1092 and 996 min for the HTK and UW groups respectively (Fig. 1). The two patients receiving their grafts after the maximum cold ischemia time are well after 10 and 17 months, respectively.

In the UW group, the patient survival rate was 74% over the whole study period, which was not statistically different from that of the HTK group.

Discussion

The study presented here is thus far the only one that compares the gold standard UW solution with Bretschneider's HTK solution for organ preservation in human liver transplantation. Bretschneider's solution is well known for its cardioplegic properties and has been in clinical use in open heart surgery for more than 15 years [2].

Although both solutions seem to be quite different at first sight, as far as their component are concerned

Table 2 Components of the current solid organ preservation solutions

[mmol/l]	HTK	UW
Na	15	30
K	10	120
Mg	4	5
Cl	50	–
SO_4	–	5
H_2PO_4	–	20/5
Histidine	180/18	–
Tryptophan	2	–
Ketoglutarate	1	–
Mannitol	30	–
Glucose	–	–
Raffinose	–	30
Lactobionate	–	100
Hydroxylethyl starch	–	50 g/l
Adenosine	–	5
Glutathione	–	3
Allopurinol	–	1
Dexamethasone	–	8 mg/l
Insulin	–	100 U/l
Osmolarity	310	320 (mosmol/l)

(Table 2) they seem to serve a similar purpose. UW solution contains lactobionate, raffinose, and hydroxyethyl starch as osmotic effective substances combined with a phosphate buffer and high potassium, as well as small amounts of glutathione, adenosine, and some other ingredients of debatable interest [1]. By comparison, HTK solution contains less potassium, but a strong histidine buffer (180/18 mmol/l) is added, which increases the osmotic effect of the mannitol also contained in this solution. The additional tryptophan, as a membrane stabilizer, hinders the histidine from entering the cells. Ketoglutarate serves as a substrate for the metabolism during the ischemic phase.

The osmolarity of both solutions is similar (310 vs 320 mosmol/l). The pk level of HTK solution is significantly higher, even during cold storage [12]. The calculated oncotic pressure of UW solution is higher (15–25 mm Hg) than that of HTK solution (0 mm Hg).

One of the requirements for optimal organ procurement is the equilibration of the preservation fluid throughout the organ. According to Bretschneider that means that the extracellular space has to be totally penetrated by the solution [3, 12]. As has been demonstrated in our pig model, a huge amount of HTK solution is required for the equilibration of the whole liver [5]. From our experience in the human adult donor, about 15–20 l of HTK solution are needed for adequate perfusion of liver, kidneys, and pancreas. Since the viscosity of HTK solution is waterlike (index 0.8 in normothermy and 3.0 at 5 °C) in comparison to UW solution (index 3 at normothermy and 9.0 at 5 °C), the length of the perfusion period is the same for both groups (10 min), despite the much larger amount

Table 3 Laboratory and clinical parameters under investigation. Values given are means. Standard deviations are indicated within parentheses

Evaluation criteria	HTK group	UW group	*P* value
Number of patients	30	30	
Bile production	24 ×	26 ×	0.397
Intraoperative bile production (ml) 1st postoperative day	142 (87)	117 (92)	0.138
Initial non-functioning	1	2 (1)[a]	0.476
Stay in ICU (days)	5.2 (16.3)	4.4 (5.3)	0.946
Use of FFP intraoperatively 1st postoperative day (bags)	11.5 (5.9)	9.9 (6.0)	0.205
Peak SGOT (U/l) 1st postoperative day	495.6 (538.3)	384.4 (398)	0.365
Peak SGOT (U/l) 3rd postoperative day	567 (1159.6)	440 (505.8)	0.156
Peak SGPT (U/l) 1st postoperative day	612.2 (605.6)	485.4 (467.5)	0.746
Peak SGPT (U/l) 3rd postoperative day	786.5 (118.3)	599.5 (585.1)	0.592
Peak GLDH (U/l) 1st postoperative day	235.6 (246.5)	198.2 (192.6)	0.357
Fibrinogen (mg%) 1st postoperative day	297.9 (43.5)	193.7 (42.9)	0.142
Fibrinogen (mg%) 3rd postoperative day	241.3 (58.9)	263.4 (78.8)	0.254
TPZ % 1st postoperative day	51.8 (17)	53.3 (10.8)	0.614
TPZ % 3rd postoperative day	64.9 (18)	69.2 (20.4)	0.217
Lactate (mg%) 1st postoperative day	2.7 (1.2)	2.4 (1)	0.797
Rejection episodes (biopsy-proven) in 1st month	13 ×	15 ×	0.584
Infection episodes (severe) in 1st month	6 ×	4 ×	0.294

[a] One liver showed delayed function

of fluid with HTK solution (20 versus 4 l). Furthermore, due to its lower viscosity, diffusion into the extracellular space is likely to occur more readily, and cooling of the organ will be accomplished in a shorter period of time. Since the amount of fluid involved is large when using HTK solution, difficulties in handling the solution may arise; however, the possible advantages of using HTK solution can offset this problem. Firstly, the solution can be released into the circulation of the recipient because of its low potassium content [13]. Secondly, HTK solution does not show any side effects after repeated perfusion of an organ [12, 13]. Thirdly, the low viscosity probably provides a better procurement of the biliary tree, something which may be supported by the observation in this study that

fewer strictures were seen in the HTK group than in the UW group, although this was not statistically significant.

From an economical point of view, 1 l of UW solution costs about DM 400.00, compared with DM 100.00 for the same amount of HTK solution. The need for about 6 l of UW solution for perfusion and storage has to be weighed against 25 l of HTK solution (DM 2400.00 versus DM 2500.00). There is a cost equivalent.

As far as the results of this study are concerned, there is hardly any difference between the two solutions in terms of their clinical use. As shown in Table 1, the mean cold ischemia time was about 9.5 h in both groups. Only seven patients received grafts in which cold ischemia time exceeded 15 h. In contrast to a previous publication [4], we did not observe any correlation between cold ischemia time and biliary complications. In the total series of 60 patients, the percentage of biliary complications was 8.3% ($n = 5$). Three of these patients died as a result of their biliary problems ($n = 1$ in the HTK group, $n = 2$ in the UW group). Colonna et al. [4] explained the severe complication of biliary strictures as a consequence of the ischemic reaction. He found a strong correlation between transaminases greater than 2500 U/l and biliary lesions. In our series these excessive reactions were only seen in the initial nonfunctioning grafts ($n = 2$). Due to the elective procedures in our series and the acceptable condition of the patients under operation (28 patients – 46% – were registered as transplantable), there were no outstanding results to be seen in either the postoperative course or laboratory data (Tables 1, 3).

There is no doubt that UW solution has changed procurement strategies and alleviated the shortage of donor organs over the last 5 years [1]. However, since the number of patients in this study is small and many factors influence organ quality and the results of organ transplantation, a multicenter trial is now necessary to shed even more light on the possible advantages of one or the other preservation solution. Theoretically and in daily practice, as presented here, preservation of the liver with HTK solution should be regarded as an alternative to preservation with UW solution. To achieve a statistically significant difference of about 5%, a multicenter trial must include at least 350 patients.

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