Transpl Int (2004) 17: 256–260
DOI 10.1007/s00147-004-0709-4

ORIGINAL ARTICLE

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Histidine–tryptophan–ketoglutarate solution for organ preservation in human liver transplantation—a prospective multi-centre observation study

Received: 7 April 2003
Revised: 3 September 2003
Accepted: 5 March 2004
Published online: 25 May 2004

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Abstract Based on experimental work and clinical small studies, histidine–tryptophan–ketoglutarate (HTK) solution was found to be suitable not only for heart and kidney preservation but also for liver preservation. We decided, therefore, to use this preservation solution for clinical liver preservation in a prospective multi-centre trial. Enrolment to the study was from 1996 to 1999 in four European centres, and the results of 214 patients with HTK-preserved organs were analysed. Analysis showed a primary dysfunction (PDF) rate of 8.8%, with a primary non-function (PNF) rate of 2.3% and initial poor function (IPF) in 6.5%. Patient survival rate at 1 year was 83% and 1-year graft survival rate was 80%. In a univariate and a multivariate analysis PDF, early surgical complications and tendentiously severe infections (septicaemia, pneumonia, cholangitis) were identified as independent risk factors for graft and patient survival. Preservation with HTK can be regarded as an established alternative to preservation with University of Wisconsin (UW) solution when preservation times are short. Definitive assessment of the efficacy of preservation solutions requires further prospective randomised clinical trials that compare HTK and UW.

Keywords Cold ischaemia time · HTK solution · Liver transplantation · Patient survival · Primary dysfunction

Introduction

The introduction of the University of Wisconsin (UW) solution in 1988 led to profound changes in the logistics of clinical liver transplantation. Wahlberg, Southard and Belzer developed a cold storage solution primarily for pancreas transplantation [1], but it was also found useful for liver preservation; the first studies were carried out by Wahlberg on liver tissue slices. In 1988 Jamieson et al. published data on preservation of the canine liver for 24–48 h, using simple cold storage with UW solution [2]. Immediately after that publication, the solution came into clinical use; Kalayoglu and colleagues from Wisconsin [3] and Todo and co-workers from Pittsburgh [4] published the first results, with extended preservation times of as long as 24 h still rendering viable grafts for transplantation.

At the Vienna transplant centre we used UW solution for the first time in June 1988; after grafting 100 livers preserved with this solution, we made our first analysis. From this retrospective analysis we concluded that we could double the preservation time for livers to a median cold ischaemia time (CIT) of 9.6 h with UW solution without compromising the graft. In 1992 Adam et al. found the impact of a CIT of less than 12 h on primary graft function, retransplantation rate, patient and graft survival [5] to be significant, criticising for their enthusiasm transplant surgeons who extended the CIT of UW-preserved organs over 12 h.

At the end of the 1980s, a further preservation solution, histidine–tryptophan–ketoglutarate (HTK) solution, originally designed by Bretschneider for cardiac preservation, entered the field of clinical transplantation [6]. This solution was found to be also suitable for ex situ operation on the liver [7], for liver preservation [8, 9, 10]; in a small randomised trial, Erhard et al. showed that both HTK and UW solution are appropriate for clinical use in liver transplantation, even if the CIT is more than 15 h [11].

In experimental settings in pigs, HTK solution was found to be comparable to UW solution for liver preservation [12, 13]. Twenty-four-hour storage of canine hepatic grafts preserved with HTK was found to be feasible, whereas 48-h storage, unlike livers stored in UW, resulted in a non-functioning graft [14]. den Butter et al. compared UW and HTK for preservation of the rabbit liver, as tested by isolated perfusion, and concluded that solutions simpler in composition than UW solution may be effective in kidney transplantation but do not appear suitable for successful liver preservation [15]. In an orthotopic rat liver transplantation model, less intracellular and interstitial oedema, fatty degeneration, intralobular necrosis and hepatocellular proliferation, and less frequent late changes, such as bile duct proliferation and vascular and sinusoidal alterations, were seen in the HTK group than in the UW group [16]. Lange et al. investigated hepatocellular injury during preservation of human livers with UW and HTK solution: they found that the effluent of HTK-preserved livers had significantly higher levels of enzyme activities than did the effluent of UW-preserved organs; as a result of the differing viscosity of the two solutions, there was better rinsing of the liver with HTK solution, although this did not necessarily indicate superior preservation by UW solution [17]. Recovery of tissue oxygenation after reperfusion in HTK-preserved livers was found to be more rapid and homogeneous than in UW-preserved livers in living-related liver transplantation [9].

We decided, like other large transplant centres (University of Pittsburgh), to use HTK preservation solution in our clinical liver transplantation programme and initiated, in May 1996, together with three further European centres, a prospective multi-centre observation trial.

The aim of this study was to investigate the influence of HTK solution on primary graft function, patient and graft survival, rejection rate and biliary complications.

Patients and methods

Between May 1996 and December 1999, four transplant centres participated in the study. The transplant unit at the University Clinic of Essen recruited 35 patients (16%), 15 patients (7%) were followed up by the University Hospital of Innsbruck, 2 patients (1%) by the University Clinic of Göttingen, and 162 patients (76%) by the University Hospital of Vienna. Follow-up data on patient and graft variables were collected prospectively 1, 3, 6, and 12 months after transplantation. Patients who underwent retransplantation and recipients under the age of 6 years were excluded from analysis.

The demographic characteristics of HTK-preserved organs and recipients are shown in Table 1. Livers were perfused simultaneously, via the aorta and the portal vein, with at least 5 l HTK for 8–10 min, in order for sufficient equilibration to be achieved. After removal of the gall bladder, the common bile duct was flushed with the HTK solution.

In all cases the operation was performed in accordance with standard techniques. In 37 patients of the

Table 1 Demographic data. Steroids were given to all patients

Characteristic	Value
Age (years)	51.6 (± 10.9)
Gender	
Male	140 (65%)
Female	74 (35%)
Primary disease	
Cirrhosis	116 (54%)
Cancers	51 (24%)
Cholestatic disease	15 (7%)
Acute hepatic failure	6 (3%)
Metabolic diseases	6 (3%)
Congenital biliary disease	0
Budd–Chiari syndrome	2 (1%)
Benign liver tumours	1
Child score	
A	32 (15%)
B	99 (46%)
C	65 (30%)
Immunosuppression	
Cyclosporin	149 (70%)
Tacrolimus	64 (30%)
Anti-thymocyte globulin	110 (51%)
Mycophenolate mofetil	17 (8%)
Azathioprine	25(12%)

HTK procurement group (17%) a veno-venous bypass was used; all vascular anastomoses were mainly completed before reperfusion of the graft. Reperfusion was carried out in 139 patients (65%) via the hepatic artery and portal vein simultaneously, in 58 patients (27%) via the portal vein, and in 13 patients (6%) via the hepatic artery first. End-to-end biliary anastomosis was performed in 187 patients (87%). Side-to-side choledochostomy was carried out in 14 patients (7%). A hepaticojejunostomy was constructed in 11 patients (5%), and a T-tube was placed in 72 patients (34%).

Graft function during the first five postoperative days was categorised into four groups: (1) good function [aspartate transaminase (AST) max $<1{,}000$ U/l and/or spontaneous prothrombin time (PT) ratio $>50\%$]; (2) fair function (AST 1,000–2,500 U/l and/or clotting factor support <2 days); (3) initial poor function (IPF) (AST $>2{,}500$ U/l and/or clotting factor support >2 days); (4) primary non-function (PNF) (retransplantation required within 7 days). Primary dysfunction (PDF) was defined as the total sum of the PNF and IPF rates.

Statistical analysis

Estimates of patient survival and graft survival were obtained with the Kaplan–Meier method. The effect of variables on patient and graft survival was analysed with multivariate analyses (Cox regression model). A P value of less than 0.05 was considered to be statistically significant. Continuous data were compared with the unpaired, one-tailed Student's t-test. Differences in group data were calculated with univariate chi-squared analysis. Mantel's and Breslow's tests were used to find differences between proportions and the significance of associations.

Results

Analysis showed a PDF rate of 8.8% (19/214) with a PNF rate of 2.3% and IPF in 6.5% of patients (Table 2). Overall patient survival rate was 91% at 3 months and 83% at 12 months; overall graft survival rate was 88% after 3 months and 80% after 1 year (Fig. 1).

Table 2 Rate of postoperative graft function

Function	Rate
Good	164 (76.6%)
Fair	28 (13.1%)
Poor (IPF)	14 (6.5%)
PNF	5 (2.3%)
Primary dysfunction (PNF + IPF)	19 (8.8%)

Rejection episodes occurred in 25.2% of HTK-perfused organs. The overall infection rate was 45% after 1 month, 52% after 3 months and 55% after the first postoperative year. Severe infections, including septicaemia, pneumonia and cholangitis, were seen in 21% (45) of patients, and local infections in 31% (66).

The incidence of biliary complications was 10% after 1 month, 17% after 3 months and 28% after the first postoperative year. Frequency of early surgical complications, including postoperative bleeding and thrombosis of V. portae and A. hepatica, was 17%, 22% and 24% at 1 month, 3 months and 12 months after liver transplantation (LTX), respectively (Table 3). Mean CIT was 444 ± 224 min.

In a univariate and a multivariate analysis, using a stepwise logistic regression model to identify independent risk factors for graft and patient survival PDF, early surgical complications and tendentiously severe infections (septicaemia, pneumonia, cholangitis) were significantly associated with poor graft and patient survival (Tables 4 and 5). No interaction or interdependence among those factors was seen. The analysis showed no positive correlation between CIT and patient or graft survival after liver transplantation. We did not observe any correlation between CIT and the occurrence of bile-duct complications.

Discussion

A multi-centre study was initiated in order to evaluate potency and safety of HTK solution. In this prospective clinical trial we documented and analysed liver procurement and preservation with HTK.

A PDF rate of 8.8% and early surgical complications were identified as independent risk factors for 1-year patient and graft survival. In an earlier study at our transplant centre we showed that IPF has a very clear-cut impact on graft and patient survival [18]. Patients with IPF showed a dramatic decrease in graft survival in the first three postoperative months and had a poor outcome at 1 year, with a 42% graft survival rate. The main cause of death of the patients with poor initial graft function was sepsis within the first 3 months after operation.

CIT had no influence on 1-year patient and graft survival, probably because the mean CIT was very short in this study. In an analysis of our data in liver transplantations carried out between 1994 and 1998, we could show a PNF rate of 1.3% in grafts with a CIT below 8.5 h, a PNF rate of 2.1% with a CIT between 8.5 h and 10 h, and a PNF rate of 6.3% in grafts beyond 10 h; we decided not to extend ischaemia time for medical reasons [19].

Furthermore, our interest in this study was focused on biliary tract complications, especially on ischaemic-

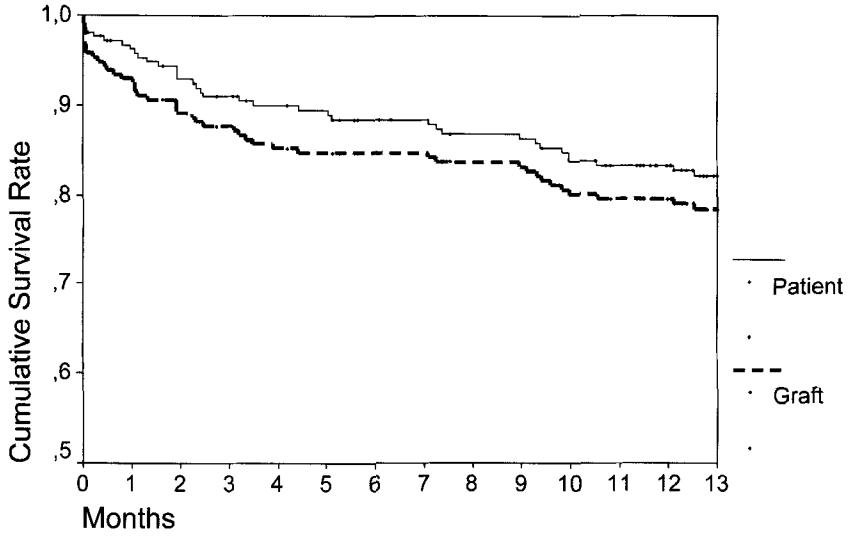

Fig. 1 Patient and graft survival rates after the use of HTK solution

Table 3 Rate of biliary complications

Biliary complication	Rate
Ischaemic-type biliary lesions	17 (8%)
Stenosis	9 (4%)
Necrosis	2 (1%)
Concrements, sludge, cast	6 (3%)
Stenosis (anastomotic)	15 (7%)
Leak (anastomotic)	9 (4%)
T-tube complications	5 (2%)
Stenosis T-tube	1
Leak T-tube	4 (2%)

Table 4 Univariate analysis (risk factors for patient survival) (*FK 506* tacrolimus, *CYA* cyclosporin, *ATG* anti-thymocyte globulin)

Factor	*P* value
PDF	0
Early surgical complications	0.001
Severe infection	0.004
CIT	0.493
FK 506/CYA	0.749
Rejection	0.821
ATG	0.96

Table 5 Multivariate analysis (risk factors for patient survival)

Factor	*P* value
PDF	0.001
Early surgical complications	0.015
Severe infection	0.018

type biliary lesions. Similar to previous publications, we did not observe any correlation between CIT and biliary complications, mainly due to the very short CIT in our series and the low rate of PDF resulting in marginally elevated transaminases (> 2,500 U/l) [20].

High-volume perfusion, suggested by Bretschneider [6] to reach equilibration of interstitial and intracellular space, is not really necessary; preservation with a total volume of 5 l for a multi-organ donor is effective and safe and yields a significant cost reduction. HTK is easy to handle, the solution is of low viscosity, and there are no substances to be added before it is used for perfusion. If tissue water content is a reliable parameter for determination of the quality of preservation, then the effectiveness of HTK solution is comparable to that of UW solution [13]. One advantage of HTK solution could be the low concentration of potassium. Theoretically, there would be no need for the preservation solution to be flushed out with an additional solution prior to reperfusion. HTK will probably be used more frequently in the future due to the lower price and ease of handling.

Beyond all preservation potency of the HTK solution, we must keep in mind that extended CIT is a risk factor adding to other risk factors of initial liver function. IPF is a major risk factor for long-term graft survival, and, consequently, patient survival. With modern preservation solutions (UW, HTK) we recommend for the liver a maximum CIT of 10 h in combination with other risk factors, and of 16 h without additional risk factors. We avoid any extension of preservation time beyond 10 h that is not medically justified.

Finally, the analysis of this multi-centre study shows that preservation of the liver with HTK solution demonstrates safety and efficacy. The preservation times in this study are not long enough to allow a useful statement to be made about long-time preservation with HTK or when injured livers from marginal donors are used. Hence, preservation with HTK can be regarded as an established alternative to preservation with UW

solution when preservation times are short. Definitive assessment of the efficacy of HTK solution requires further prospective randomised clinical trials that compare HTK and UW [11].

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