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Comparison of HTK- and UW-solution for liver preservation tested in an orthotopic liver transplantation model in the pig

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Abstract. The aim of this experimental study was to compare the preservation potency of University of Wisconsin (UW) and HTK (Bretschneider) solutions in an orthotopic liver transplantation (OLT) model in pigs. Livers were harvested using an in situ perfusion technique, where organs were flushed with the solution being tested, stored on ice – cold storage (CS) – for 2 or 24 h and then transplanted. Parameters monitored were liver enzymes in serum, hepatic water content, high energy phosphates, nuclear magnetic resonance (NMR) relaxation time T2, light microscopy and bile production. CS for 24 h is an extreme in pig liver preservation and is not compatible with animal survival. Biopsies showed drastic morphological changes and grafts did not produce bile in either group. (Bile production 2 h CS: HTK, 5.6 ± 1.8 ml/h; UW, 4.7 ± 2.3 ml/h) Enzyme release after reperfusion (ΔSGOT, ΔLDH) was higher in long-term preservation. Hepatic tissue water content significantly decreased during CS in UW preserved livers. Edema alter reperfusion (ΔH_2O: HTK 24 h = + 5.6%, UW 24 h = + 4.8%) and regeneration capacity after reperfusion (UW 2 h = 63%, HTK 2 h = 55%, UW 24 h = 30%, HTK 24 h = 30%) were not significantly different. However, we did not observe major differences in preservation potency between the solutions tested. Differences were correlated, rather, with length 9 time of CS, than with the solution used. Therefore, HTK solution seemed to be a low potassium containing alternative to UW solution.

Key words: Comparison of HTK and UW solution – Liver preservation – Liver transplantation – Pig

The introduction of University of Wisconsin (UW) solution in solid organ preservation has had a major impact, particularly on the logistics of liver transplantation. Since this solution permits the storage of canine livers for 48 h [8] and human livers for 24 h [16], orthotopic liver transplantation has changed from an emergency procedure to a semielective procedure and, therefore, UW solution is currently standard in clinical liver transplantation. However, the histidine-buffered tryptophan ketoglutarate solution of Bretschneider (HTK), originally designed for cardioprotection and in clinical use for many years in heart surgery, has recently entered the field of solid organ preservation. Clinical trials are presently being carried out on its use in preservation of the kidney [3] and recent reports claim this solution is also very effective in preservation of the liver [13]. The results of organ preservation in the cold for these two solutions are quite different. One key component in UW solution seems to be the appropriate concentration of impermeants, counteracting hypothermic-induced cell swelling, whereas HTK solution contains very effective buffer systems, using the principal of equilibration of the extracellular space without any impermeants. The aim of this experimental study was to compare some aspects of the preservation potency of UW and HTK solutions in short- (2 h) and long-term (24 h) cold storage (CS) in an orthotopic liver transplantation (OLT) model in the pig. Efficacy of preservation was evaluated by monitoring the following parameters during the procedure: histological examination of liver specimens, hepatic tissue water content, high energy phosphate levels, graft nuclear magnetic resonance NMR relaxation time T2, enzyme release and bile production after reperfusion.

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Materials and methods

Young, white large pigs of the German land race weighing 20–25 kg (mean 22.5 ± 2.6 kg) of either sex were used as donors and recipients. After a fasting period of 24 h, livers were harvested from the donors using an in situ perfusion technique. Organs were flushed by gravity with the test solution (4 °C) by giving 1 l via the aorta (pressure = 120 cmH_2O) and 1 l via the portal vein (pressure = 25 cmH_2O). HTK preserved organs were flushed with an additional 2 l via the portal vein at the backtable, according to the recommendation of the manufacturer, to achieve equilibration of the extracellular space.

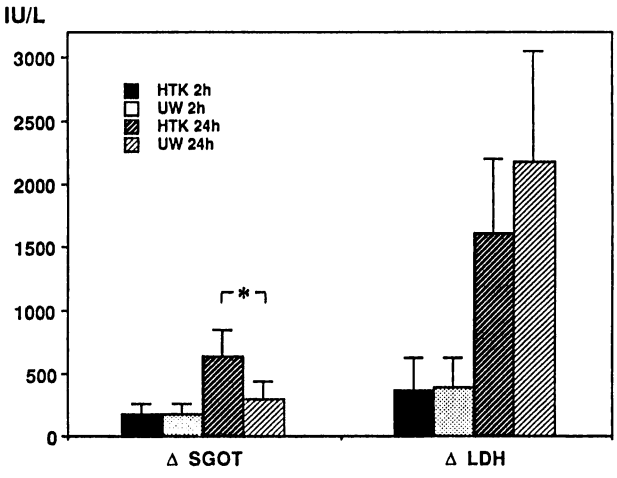

Fig. 1. Changes in SGOT and LDH after reperfusion (IU/l). Significant changes in ΔSGOT and ΔLDH in short- and long term HTK, and in ΔLDH in short- and long-term UW preserved grafts. There was a smaller ΔSGOT in 24 h UW preserved liver (* $P < 0.05$) as compared to 24 h HTK preserved livers

The livers were stored in an additional 500 ml of the same solution in two sterile plastic bags, packed on ice (0–4 °C) and subjected to a short or a long time of cold storage (CS). Groups were as follows: short-time preservation (CS = 2 h): HTK $n = 3$, UW $n = 4$; long-time preservation (CS = 24 h): HTK $n = 6$, UW $n = 6$.

At the end of the preservation period, the livers were transplanted orthotopically using a standard technique. A passive veno-venous bypass from the end of the portal vein to the internal jugular vein was used during the anhepatic period. Before completing the infrahepatic caval anastomosis, all grafts were washed out via the portal vein with 500 ml Haemaccel to remove the preservation fluid. This step was necessary only in UW preserved livers containing high levels of potassium, but was done also in HTK preserved organs to keep experimental conditions comparable. Reperfusion of the liver was carried out simultaneously via the hepatic artery and the portal vein. Hemodynamic conditions were monitored by continuous registration of CVP and arterial blood pressure. Animals with hemodynamic instability, especially during reperfusion, were excluded. Blood samples (collected from a catheter in the jugular vein) and liver excision biopsies were obtained at the beginning of the donor operation (normal), at the end of cold storage (CS) before and after the Haemaccel flush, immediately before reperfusion (WIT), and 30 min and 2 h after reperfusion. For clarity, only the data of three points of greatest interest during the procedure were compared: normal, end of CS and 30 min after reperfusion. Liver enzymes in serum were estimated by using comercially available kits. For histological examinations, liver biopies were fixed in 4.5 % formol, prepared for light microscopy and stained with HE and CAB. Bile was collected quantitatively by insertion of a catheter into the common bile duct of the graft. For estimation of overall hepatic tissue water content, liver specimens were blotted and weighed immediately after excision (mean wet weight of sample 27.8 ± 9 mg) and then reweighed again after drying at 90 °C for 36 h. Hepatic tissue water content is given as the difference between wet and dry weight of liver specimens. High energy phosphate levels of liver tissue were assessed by high performance liquid chromatography (HPLC). In preparation for HPLC analysis using an LKB HPLC system (LKB-Produktor AB, Bromma, Sweden), tissue samples were weighed and immediately homogenized in 3 ml of 8 % perchloric acid [4]. The homogenate was centrifuged for 10 min at 3000 rpm, the supernatant was collected and adjusted to pH 6.0 using 4 M KOH. After passing through a 0.45 µm acro-disk filter (Sartorius Göttingen, FRG), 20 µl of the sample was applied to a reverse phase column (LKB 2134 UltroPac Li-Chromosorb RP-18.5 µm 4.0 × 250 mm) and eluted at a flow speed of 0.5 ml/min with 0.2 M NaH_2PO_4, 0.025 M tetrabutylammoniumhydroxyde, 18% methanol (v/v), pH 6.0. Peaks were detected at 254 nm by an ultraviolet detector (LKB 2151 variable wavelength monitor) and recorded on a LKG 2210 recorder. Each peak was assigned by comparison with retention times of authentic compounds. The concentration of nucleotides was estimated by measuring the peak area. Energy charge (EC) was calculated according to the formula by Atkinson [1]. Proton nuclear magnetic resonance (NMR) spin-spin relaxation time T2 was determined 30 min after biopsy excision using a low resolution1H-NMR spectrometer (Minispec pc 120, 0.47 T Bruker, Karlsruhe, FRG) and applying a CPMG pulse sequence (TE = 2 ms, N = 100, Tr = 3.0 s, 9 averages) [11]. Measurement temperature was 37 °C. A monoexponential model was fitted for quantitative data analysis.

Statistical analysis. Mean values and standard deviations are given in the results section. Differences were analysed by means of *t*-test or by Mann-Whitney U test (nonparametric data). Probability values of $P < 0.05$ were considered to be statistically significant.

Results

Histological examinations. In all cases, we could differentiate clearly between long- and short-term preservation, because of major differences in the morphological findings after reperfusion. However, it was impossible to find major differences between organs preserved in HTK or UW. When the length of CS was 2 h, all specimens (UW and HTK) showed a normal hepatic architecture with normal sinusoidal structure at the end of CS. Also on reperfusion, there was no evidence for the occurrence of severe hemorrhage or detachement of endothelial cells. After 24 h of CS in both groups (UW and HTK), a regular organisation of the hepatic lobules and considerable sinusoidal dilatation with pyknosis of some sinusoidal endothelial cells was observed. Hepatocytes were quite intact, except for the occurrence of moderate microvesicular steatosis. After flushing the grafts with Haemaccel to remove the preservation fluid, a marked detachment of sinusoidal lining cells with accompanying enlargement of the spaces of Disse was seen. At 30 min after reperfusion, drastic morphological changes were obvious in liver specimens from both groups. The hepatocytes were spread by sinus' full of blood, with pyknosis of the nucleus in several cells, there was necrosis of numerous single cells and areas with hepatocyte apoptosis. In addition, we found considerable areas of hemorrhage in the portal tracts and even in the walls of hepatic veins. Numerous sinusoidal lining cells were detached from the underlying hepatocytes with condensed nuclei and pyknosis.

Bile production. Long-term preserved grafts did not produce any bile in the first h after reperfusion after preservation in either UW or HTK. Short-term preserved organs immediately produced comparable amounts of well-colored bile in both groups. (HTK, 5.6 ± 1.8 ml; UW, 4.7 ± 2.3 ml).

Liver enzymes. Changes in GOT and LDH are depicted in Fig. 1 as the differences between concentrations 30 min after reperfusion and normal values. There were significant differences in ΔSGOT and ΔLDH between short-

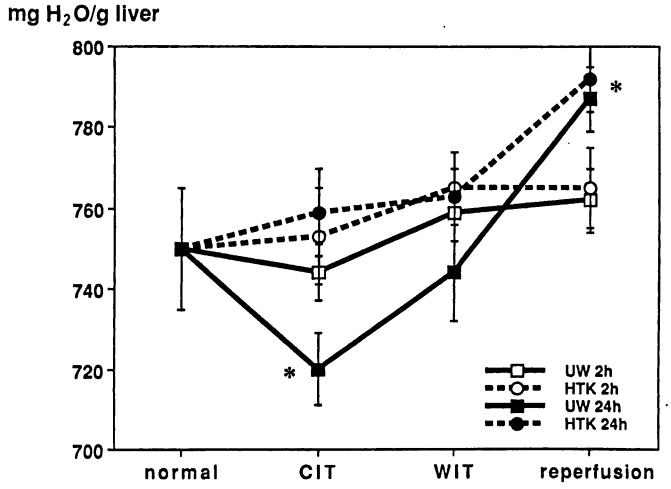

Fig. 2. Hepatic tissue water content at the end of cold storage *(CIT)*, immediately before *(WIT)* and 30 min after reperfusion (mgH_2O/g liver). There was significantly (* $P < 0.05$) less water content in 24 h UW preserved grafts at the end of CIT. There was marked edema after reperfusion in all livers stored for 24 h (* $P < 0.05$)

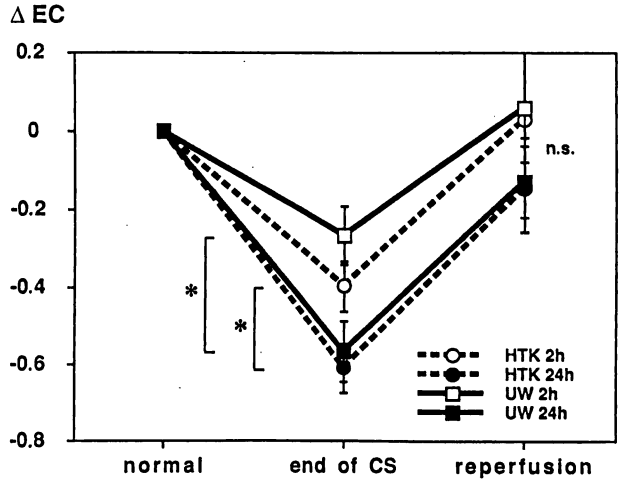

Fig. 4. Changes in energy charge *(EC)* of liver tissue at the end of CS and after reperfusion. EC = ATP + 0.5 ADP/ATP + ADP + AMP. There were significantly different EC levels at the end of CS between long- and short-term preserved grafts (* $P < 0.05$). There was good restoration of EC after reperfusion in grafts stored for 24 h. No major effect of the solution used was observed

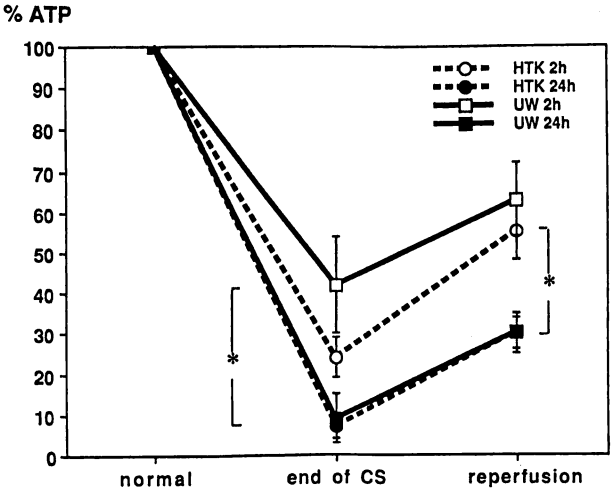

Fig. 3. ATP (% of normal) in liver tissue at the end of CS and after reperfusion. There was significantly less ATP in long-term preserved grafts after CS and after reperfusion (* $P < 0.05$). No major effect of the preservation solutions was observed

and long-term preservation in HTK (ΔSGOT, 174 ± 86 IU/l vs 639 ± 207 IU/l; ΔLDH, 363 ± 264 IU/l vs 1611 ± 590 IU/l; $P < 0.01$) and in ΔLDH between short- and long-term preservation in UW (383 ± 239 IU/l vs 2179 ± 869 IU/l, $P < 0.01$), but no significant difference was observed in ΔSGOT levels between UW (179 ± 82 IU/l vs 297 ± 133 IU/l). Comparison of UW and HTK solutions for the same preservation time showed no differences in enzyme release despite lower ΔSGOT levels in the 24 h UW group as compared to the 24 h HTK group ($P < 0.05$).

Tissue water content. In organs preserved for 2 h, hepatic tissue water content was found to be in the normal range in the HTK as well as in the UW group at the end of CS and also after reperfusion (Fig. 2). Normal hepatic tissue water content was found to be 750.8 ± 16 mg H_2O/g liver. Long-term UW preserved livers showed a significant decrease in tissue water content at the end of CIT (720.5 ± 9.5 mg H_2O/g liver; $P < 0.01$ compared to normal), whereas in long-term HTK preserved organs no major changes were detectable after 24 h of CS (763.2 ± 7.8 mg H_2O/g liver). There was a significant difference between the water content of HTK preserved organs and UW preserved organs at the end of CS. Marked tissue edema, both in UW and in HTK preserved grafts (789.2 ± 8.7 mg H_2O/g liver vs 792.2 ± 8.3 mg H_2O/g liver, $P < 0.01$ compared to normal) was observed after reperfusion with no significant difference between the groups.

High energy phosphates. Due to the large scatter of data obtained from normal pig liver (ATP, 7.26 ± 0.94 μmol/g dry liver tissue; EC, 0.719 ± 0.063), the percentage of normal values (for ATP) and differences from normal values (for EC) were used for comparison between the groups and results are shown in Figs. 3 and 4. After 2 h of CS, ATP levels dropped to 24.1 ± 5% in HTK and to 42.2 ± 12% in UW preserved livers ($P < 0.05$) and were significantly lower ($P < 0.05$) after 24 h of CS in HTK (6.9 ± 3.2%) and UW preserved grafts (9.2 ± 5.5%). On reperfusion of the organ, ATP levels increased in short-term preserved organs to 54.8 ± 7.3% (HTK) and 62.7 ± 9.4% (UW) and in long-term preserved organs to 30.1 ± 4.1% (HTK) and 30.0 ± 5.5% (UW), indicating significant differences ($P < 0.05$) only for the length of preservation and not for the solution used. Energy charge dropped by 0.39 ± 0.06 (HTK) and 0.27 ± 0.07 (UW) after 2 h and by 0.61 ± 0.06 (HTK) and 0.57 ± 0.08 (UW) after 24 hours CS. After reperfusion EC returned to normal in short-term and to near normal values in long-term preserved organs. These changes were not significant.

Spin-spin relaxation time T2. Due to the large scatter of data obtained from normal pig liver (T2 = 46.5 ± 4.1 ms)

only the use of Δ values was appropriate. At the end of CS, long-term UW preserved livers showed a marked decrease in T2. There was no change in the HTK group. (HTK 2 h, -1.9 ± 3.3 ms; HTK 24 h, -0.34 ± 4.9 ms; UW 2 h, -1.1 ± 3.4 ms; UW 24 h, -8.2 ± 2.9 ms). There was a highly significant correlation between ΔT2 and Δ water content (HTK, $r = 0.80$; UW, $r = 0.85$; $P < 0.001$) for both storage solutions. Also highly significant correlations were detected between ΔT2, ΔATP and Δ EC for UW. (ΔT2/ΔATP, $r = 0.75$, $P < 0.005$; ΔT2/ΔEC, $r = 0.73$, $P < 0.005$).

Discussion

In a review of the principles of solid organ preservation [2] Belzer and Southard have suggested, that an effective and appropriate flushout solution should have a composition that (1) minimizes hypothermic induced cell swelling, (2) prevents intracellular acidosis (3) prevents the expansion of the interstitial space during the flushout period, (4) prevents injury from oxygen free radicals and (5) provides substrates for regenerating high-energy phosphate compounds during reperfusion. The aim of our study was to evaluate points (1), (3), and (5) of these principals, determining tissue water content and high energy phosphate levels in an experimental model in the pig. Using a large animal model, the conditions of human liver transplantation were well imitated, with similar operative trauma, a second warm ischemic period during grafting and similar sized vessels for anastomosis. Furthermore, we examined time-periods during the procedure, where the quality of the preservation solution had a major impact on liver viability and the influence of factors unrelated to preservation were minimal. Therefore, this model should be suitable for the comparison of preservation solutions. Looking at morphology and bile production after reperfusion, there was marked damage to the grafts stored for 24 h as early as 30 min after reperfusion, whereas the appearance and function of livers stored for 2 h was quite normal. Furthermore, tissue edema, as monitored by hepatic water content, indicated considerable disturbance of cell membrane integrity. In this study CS for 24 h seemed to result in liver damage independent of the protective effect of the solution used. However, a certain regeneration capacity of high energy phosphates was also obvious in long-term preserved grafts, as shown by an increase in ATP and EC levels after reperfusion. Hepatocytes are thought to be relatively resistant and to be involved only secondarily in ischemia-reperfusion injury [17]. Good energy restoration early after reperfusion and minimal changes in cell-morphology of the hepatocytes confirmed these findings. Preventing micro-circulatory disturbances by maintaining the integrity of endothelial and sinusoidal lining cells during CS appears to be very important for liver preservation [7, 12]. Several reports have been published concerning damage to endothelial cells by using flushout solutions for organ preservation [9, 10]. In our study, in both groups (HTK and UW), after 24 h of CS, the first signs of injury were already evident in sinusoidal endothelial cells at the end of CS. Looking for a quick viability parameter to distinguish, before reperfusion, between good and poor donor livers we investigated T2 relaxation times, and found correlations with tissue water content and high energy phosphate levels. However, these promising results were obtained from a small series and the value of this method has to be confirmed in further studies. Most of the parameters monitored in our study could clearly differentiate between short- and long-term preservation. Comparing HTK and UW solution by means of hepatic water content, it was evident that in grafts preserved in HTK, tissue water content remained in the normal range even after long-term preservation; UW solution was able to draw water from the tissues during CS, but edema after reperfusion was also found to be in the normal range. Although UW solution contains adenosine, which has been found to be an effective substrate for the resynthesis of ATP, we did not observe any major differences in the regeneration capacity of high energy phosphates in our model. The effectiveness of adenosine was demonstrated by using isolated cell models of organ preservation and the importance of adenosine in UW solution is still debated [14]. Comparing HTK and UW solutions in the preservation of the pig liver, we did not observe a major advantage in using one or other of the solutions. A solution that offers greater buffering capacity and uses the principle of equilibration would have greater preservation potency. This would be achieved by a solution stressing the osmotic force during CS. First attempts to combine histidine and lactobionate in a new solution are reported to be very effective [15]. Therefore, the HTK solution seems to be a low potassium containing alternative to the UW solution. The first promising results in clinical liver preservation with HTK solution confirm our results [5, 6].

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