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The impact of liver preservation in HTK and UW solution on microcirculation after liver transplantation

F. Walcher, I. Marzi, and V. Bühren

Department of Surgery, University of Saarland, Homburg/Saar, Germany

Abstract. Severe microcirculatory disturbances due to endothelial cell damage and leukocyte adherence during reperfusion of transplanted livers are considered to contribute to early graft failure. Since the degree of reperfusion injury after liver transplantation depends on the length of preservation time and the solution used for preservation, the aim of our study was to assess three solutions with respect to microvascular perfusion and leukocyte adhesion. Therefore, rat livers were stored up to 24 h in Euro-Collins (EC), University of Wisconsin (UW), or histidin-tryphtophan-ketoglutarate (HTK) solutions prior to orthotopic transplantation. The livers were studied in situ 60 min postoperatively using intravital fluorescence video microscopy. Using simple syringe flushing (10 ml), sinusoidal perfusion decreased below 50% in EC preserved livers after 8 h preservation, in HTK preserved livers after 16 h preservation, and remained higher than 70% in livers preserved in UW up to 24 h. Permanent adhesion of leukocytes was increased more rapidly in organs after 1, 8, 16, and 24 h preservation in HTK (16%, 15%, 34%, and 49.7% ± 4.7%) compared to those preserved in UW (15%, 18%, 17%; and 32.7% ±3.3%; $P < 0.05$). Using a 10-fold volumn of the organ weight of HTK solution during the harvesting procedure, with an 8 min equilibration period, sinusoidal perfusion (39.6 ± 4.7%) and leukocyte adhesion (42.7 ± 3.1%) were not improved after 24 h. In contrast, equilibration with a volumn of approximately 40-times the liver weight improved sinusoidal perfusion (70.8% ± 2.7%; $P < 0.01$) and leukocyte adhesion (24.9% ± 3.1%; $P < 0.01$) significantly. Thus, using HTK solution, simple flushing prior to long-term cold storage resulted in microcirculatory disturbances when compared to UW solution. Larger volumns of HTK solution with an additional equilibration period of 8 min, however, reduced leukocyte adhesion and improved sinusoidal perfusion to a similar degree as UW solution.

Key words: Liver transplantation – Preservation solutions – UW solution – HTK solution – Leukocyte adherence – Microcirculation

Reperfusion injury to sinusoidal endothelial cells and microcirculatory disturbances have been observed after liver transplantation in recent years [3, 9, 11]. Subsequent adhesion of leukocytes leading to organ injury as well as microcirculatory perfusion failure were considered to contribute to primary nonfunction or poor function of liver grafts [19]. The degree of reperfusion injury and microcirculatory disturbances, however, depend on the period of cold storage prior to transplantation and the preservation solution used. In the late 1980s, the University of Wisconsin cold storage solution (UW) [1] was introduced and has replaced Euro-Collins solution in many centers allowing significantly longer preservation times [13]. In 1990, histidine-tryphtophane-ketoglutarate solution (HTK), developed by Bretschneider [2], was used successfully for clinical liver transplantation [4].

A comparative study using intravital fluorescence microscopy after rat liver transplantation by our group has shown recently that UW and HTK solutions comparably reduce microcirculatory disturbances, and both are superior to Euro-Collins (EC) solution [10]. This study, however, was performed after cold storage of livers for 1 h in a standard liver transplantation model. The aim of the present study was, therefore, to assess microcirculation and leukocyte adhesion in transplanted rat liver after cold storage periods of up to 24 h.

Materials and methods

Fed female Lewis rats (HAN, Hanover, FRG) weighing 220–250 g were used as donors and recipients to exclude immunological interference (n = 3–7/group). In the first part of the study, the livers were harvested after flushing the organs with 10 ml of ice-cold Euro-Collins (Fresenius, Bad Homburg, Germany), UW (DuPont, Waukegan, Illinois, USA), and HTK (Custodiol, Dr. F. Köhler Chemie, Alsbach, Germany), respectively (syringe flushing). The

Offprint requests to: I. Marzi, Dept. of Surgery, University of Saarland, D-6650 Homburg/Saar, FRG

organs were stored in the cold for 1 to 24 h, except those in EC solution which were stored only for 1 and 8 h. Since equilibration with HTK solution for at least 6 min has been suggested by Bretschneider [2], we modified the havesting procedure in the second part of the study. In two additional groups, livers were perfused for 8 min with a volumn of 10–12 times the liver weight or 40–45 times the liver weight. To keep all other experimental conditions constant (e.g., equilibration time of 8 min), the larger volumn was applied by elevating the bottle of the cold storage solution (40 cm and 100 cm, respectively). To prevent rewarming of the solution during perfusion, a cooling system was used.

All livers were transplanted orthotopically according to the technique described earlier [6]. The rats were anaesthetized with pentobarbital sodium (30 mg/kg i.p.) 60 min after surgery, and the liver was exposed under an intravital microscope (Nikon MM1; Düsseldorf, Germany; 545 nm filter, 20× water immersion objective) achieving a final magnification of 330×, as described recently [9]. After injection of the leukocyte marker, acridine orange (1 µmol/kg; Sigma Chemicals, Deisenhofen, Germany), 5–8 pericentral fields of the liver lobule were recorded continuously for 30 s using a CCD camera (Cohu, FK 6990, Fa. Pieper, Schwerte, Germany) and an S-VHS video recording system (Panasonic, NV-FS1, Japan). Using off-line frame-by-frame analysis, sinusoidal perfusion and adhesion of leukocytes were analysed. Data of permanently adherent leukocytes, defined as white blood cells adhering at least 20 s to the sinusoidal wall, were given as percentage of all labelled and observed leukocytes [10].

Results

The hepatic microcirculation was disturbed in all groups as reflected by the decrease in sinusoidal perfusion (Table 1). The sinusoidal perfusion rate declined to below 50% in the EC solution group after 8 h preservation and in the HTK solution group after 16 h preservation, while UW preserved livers still had a perfusion rate above 70% after 24 h of cold storage. Adhesion of leukocytes, which

Table 1. Microcirculation and permanent leukocyte adhesion after cold storage in EC, HTK and UW as investigated 1 h after liver transplantation

Solution	EC		HTK				UW			
Storage time (hours)	1	8	1	8	16	24	1	8	16	24
Microcirculation (%)	84	47	89	83	59	37	93	81	74	73
Leukocyte adhesion (%)	34	45	16	15	34	50	15	18	17	33

was significantly increased after 8 h of cold storage in EC solution, rose substantially in livers stored for 16 h in HTK and in livers stored for 24 h in UW. Thus, cold storage with HTK solution after simple flushing with 10 ml resulted in a significant reduction in sinusoidal perfusion and an increase in adhesion of leukocytes compared with UW solution under identical conditions (Figs. 1 and 2). A larger volumn of HTK solution (approx. 10× liver weight) did not improve sinusoidal perfusion (Fig. 1) and leukocyte adhesion (Fig. 2). However, high volumn equilibration (approx. 40× liver weight), allowed a significant improvement of the microvascular perfusion (Fig. 1) and attenuation of leukocyte adhesion (Fig. 2). Using this procedure, the data were comparable with those of the UW group with no statistical difference.

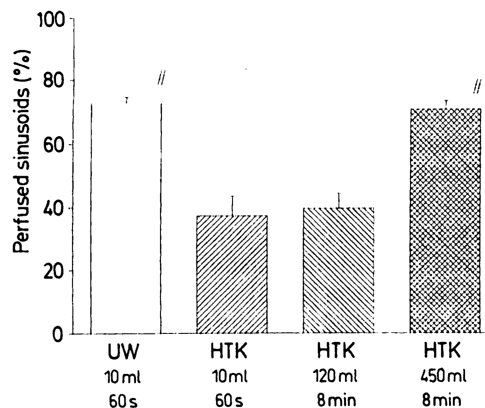

Fig. 1. Sinusoidal perfusion after 24 h of cold storage in UW and HTK solution. Application procedure as indicated and described in Methods. Data are expressed as mean ± SEM. #, $P < 0.05$

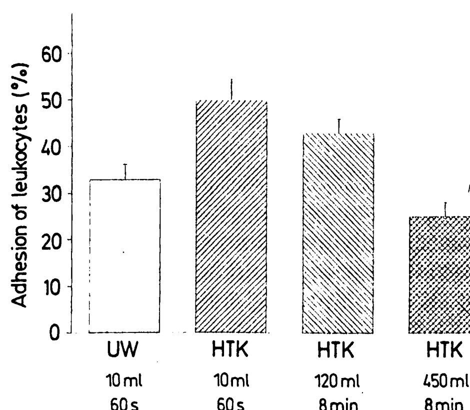

Fig. 2. Leukocyte adhesion after 24 h of cold storage in UW and HTK solution. Application procedure as indicated and described in Methods. Data are expressed as mean ± SEM. #, $P < 0.05$

Discussion

During reperfusion of liver grafts, endothelial cell injury has been demonstrated [3, 11] and has been suggested to cause microcirculatory failure of the transplanted organ [19]. On the other hand, activation of liver macrophages has been demonstrated after cold storage and transplantation of rat livers [18]. The macrophages most likely release inflammatory mediators (e.g., PAF, leukotrienes) deteriorating further microvascular perfusion [20]. Microvascular perfusion failure is due partly to an increased expression of adhesion sites (e.g., GMP140, ELAM-1, ICAM-1) on endothelial cells [14] and can be induced by inflammatory mediators generated during the reperfusion period [7]. Subsequently, activated leukocytes emigrate into the tissues, and organ destruction can be expected [8]. Indeed, increased adhesion of leukocytes and microcirculatory disturbances have been shown after rat liver transplantation using in vivo fluorescence microscopy techniques, even after 1 h of cold storage in EC, UW, and HTK solutions [10]. Since long-term preservation is becoming more important clinically, the aim of this study was to evaluate its effect on hepatic microcirculation after transplantation.

The results of this study demonstrated clearly the superiority of UW and HTK solution over EC solution after

liver preservation up to 8 h with respect to sinusoidal perfusion and number of adherent leukocytes. This is consistent with other experimental and clinical studies comparing UW solution in liver [12, 13, 17] and HTK solution in kidney transplantation with Euro-Collins solution [5]. The benificial effects have been attributed in part, to prevention of cell swelling and free-radical-mediate reperfusion injury by impermeant and antioxidant ingredients [1, 15] and the histidine/histidine chloride buffering system in HTK solution [2, 16].

The second part of the study demonstrated the importance of different application procedures using UW and HTK solution. Whereas organ protection with UW was achieved with simple flushing (10 ml) only, HTK solution required equilibration with a high volumn for 8 min to achieve comparable results (Figs. 1, 2). This method of application of HTK solution was based on the extensive studies of Bretschneider [2]. He noted that the extracellular fluid in the HTK solution has low potassium levels, which facilitates flushing by the prevention of potassium induced vasoconstriction. Moreover, the viscosity of the HTK solution is lower than that of the UW solution, containing hydroxyl-ethyl starch as impermeant [1]. To achieve an equilibration of the extracellular space, a volumn of 20 times the organ weight with a pressure of 120 cm was used successfully for kidney protection [2]. In clinical liver transplantation with cold ischemia times up to 12.5 h, 9–10 l HTK solution were given via the portal vein and the aorta for 6–8 minutes with good results [4]. In this experimental study, HTK solution was only comparable with UW solution when a substantially higher volumn was used. The reasons why such a large volumn was needed to reduce microcirculatory failure significantly in this animal model remain unclear. This needs to be evaluated in further studies.

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