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Comparison of University of Wisconsin (UW) and Eurocollins (EC) preservation solutions in a rat liver transplant model

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Abstract. The Eurocollins (EC) and University of Wisconsin (UW) preservation solutions were compared in a rat liver transplant model. After hepatectomy, 48 rat livers were flushed with either EC or UW preservation solution and were randomly assigned to 1, 12, 24, and 30 h of preservation at 4 °C, resulting in eight groups each containing six livers. Following preservation, orthotopic liver transplantation with reconstruction of the hepatic artery was performed. The efficacy of the preservation solution was assessed at 48 h post-transplantation by survival histological features and aspartate transaminase assay (AST) values. None of the rats survived 30 h of liver preservation with EC whereas five out of six rats did with UW preservation. After 24 h of liver preservation, three of the six rats in the EC group survived, compared to all six rats in the UW group. Histological evidence of severe ischemia was found in both groups in all but one survivor (UW, 24 h). After 12 h of EC preservation, one rat died within 48 h and severe ischemic changes were found in the remaining five rats. Among the rats with 12 h of UW preservation, only two out of six showed ischemic changes, and all six rats survived beyond 48 h. Without preservation (1 h), ischemic damage was found in two out of six rats in each group and all rats survived. The median AST values were higher in the EC groups than in the UW groups; the difference became significant after 12-h preservation (EC 900 IU/l versus UW 465 IU/l) and 24-h preservation (EC 5220 IU/l versus UW 631 IU/l). However, the median AST value in the five surviving rats whose livers had been preserved for 30 h in UW climbed to 1880 (950–2240) IU/l. We conclude that UW solution provides better long-term preservation than EC solution. However, even with UW solution, the observed mortality, the severity of ischemic changes, and the pronounced increase in the median AST value cast doubt upon the safety of liver preservation beyond 24 h.

Key words: Preservation, liver, rat – UW versus Eurocollins solution, liver preservation – Liver preservation, in the rat

Since the early 1970s, preservation research has been overshadowed by the fascinating developments in immunology, particularly in the treatment of rejection. In contrast, no further evolution has taken place in the methods of organ preservation, namely cold storage or, less frequently, machine preservation. Since 1979, Eurocollins (EC) solution [3], a simplified version of a solution for kidney preservation developed by Collins et al. [2], has been used as a "universal" preservation solution, allowing human kidneys to be stored for 36–48 h and human livers for 10–12 h [1].

In 1986, a conceptually new preservation solution was developed at the University of Wisconsin (UW solution) [13]. This solution, initially designed for pancreas preservation, proved to be very effective in liver preservation as well. In the dog, successful preservation of the donor liver up to 48 h has been reported [7], while in human liver transplantation, preservation has been reported to be successful up to 24 h [8]. Todo et al. [12] stated that in 185 liver homografts preserved with UW solution, compared to 180 liver grafts preserved with EC solution, the preservation time could be extended from 9.5 h with EC solution to 24 h with UW solution with equal survival and less primary graft failure. However, we noticed that the median preservation duration was about 7 h for both groups and that the transaminase levels in the patients with grafts preserved between 15 and 24 h tended to be higher than those of grafts preserved for 10 to 15 h. We are not aware of an experimental or controlled clinical

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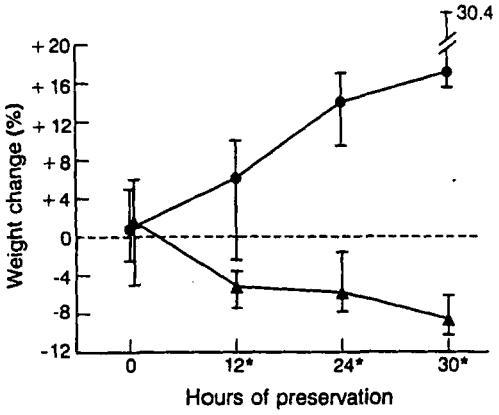

Fig. 1. Weight changes (in %) of hepatic allografts in rats in relation to duration of preservation with EC (•) and UW (▲) solutions (median values and range). * $P < 0.05$ (Mann-Whitney U-test)

trial in liver transplantation comparing EC and UW solutions. Therefore, we decided to perform a controlled trial to compare EC and UW solutions with different durations of preservation in our liver transplant rat model [11]. A unique feature of this model is the successful reanastomosis of the hepatic artery, which leads to fewer post-transplant complications and, thus, more uniform results.

Methods

The technique of orthotopic liver transplantation in the rat with rearterialization of the graft is described in detail elsewhere [10]. Male syngeneic Lewis rats weighing approximately 250 g were used. Except for 2.5 µg atropine for premedication, no other drugs or supplemental oxygen were used before or after transplantation. In all, 60 rat liver transplantations were performed; 48 animals were included in this study. In 12 rats the procedures were technical failures; that is, there was thrombosis of the hepatic artery in 8 rats, thrombosis of the portal vein and the vena cava in 1 rat each, and 2 perioperative deaths. The arterial anastomosis failures were evenly distributed among the experimental groups and were not followed by premature death of the recipient animal.

The 48 donor livers were flushed in situ via the portal vein with 5 ml EC or UW solution at 4 °C and were randomly assigned to eight groups with 1, 12, 24, and 30 h of preservation time; in other words, each group contained six livers flushed with either solution. The weight of the liver was determined immediately after removal from the donor rat (baseline weight) and after the preservation period prior to implantation into the recipient rat. The weight change after preservation at 4 °C was expressed as a percentage of the baseline weight. Autopsy was performed after premature death or after 48 h post-transplantation in the surviving rats. The 48-h follow-up period was chosen to obtain data at the time of anticipated maximal aspartate transaminase assay (AST) and histological changes. The graft was assessed for the absence of technical failures. After blood for AST was aspirated from the heart, the surviving rats were sacrificed and the livers were removed for histological assessment. Liver damage was graded histologically from 0 (normal) to 3 (widespread ischemic necrosis).

Results

Survival

Thirty-seven of the 48 rats included in the study survived until the protocol autopsy after 48 h postoperatively (Table 1); the deaths were not caused by a technical problem. Ten of these 11 deaths were in the EC groups; all 6 rats with 30 h of liver preservation died, 3 of the 6 rats in the 24-h liver preservation group and 1 of the 6 in the 12-h liver preservation group died within 48 h post-transplantation. The only death in the UW groups occurred in the 30-h liver preservation group.

Weight change

The effect of the EC or UW solution on the weight of the donor liver during preservation was different (Fig. 1). Using EC solution, the liver gained weight progressively with the duration of preservation, reaching a median increase of 17.1% after 30 h of preservation. In contrast, with UW solution, the liver weight decreased up to a median of 8.6% after 30 h of preservation compared to an increase of 1.6% after 1 h of preservation. Except for the findings after 1 h of preservation, the differences in weight change between the EC and UW groups were significant.

Table 1. Survival, histologic changes, and changes in aminotransferase (AST) levels in rat liver allografts after preservation with Eurocollins (EC) and University of Wisconsin (UW) solutions

Duration (hours)	1		12		24		30	
Solution	EC	UW	EC	UW	EC	UW	EC	UW
Number	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$	$n=6$
Survival >48 h	6	6	5	6	3	6	0	5
Histology								
Grade[a] 1	4	4	–	1	–	–	–	–
2	1	–	2	5	–	4	–	2
3	1	2	3	–	3	2	–	3
AST[b] (IU/l)	539	127	900[c]	465	5220[c]	630	–	1940
Range	187–960	121–1890	540–6500	243–1120	3020–7150	505–2800	–	940–6950

[a] Grade 1 = near normal to mild changes; 2 = moderate ischemic damage; 3 = severe ischemic damage
[b] Median normal AST value = 105 IU/l
[c] $P < 0.05$ (Mann-Whitney U-test) EC compared to UW in the 12 recipients in the 24-h storage group

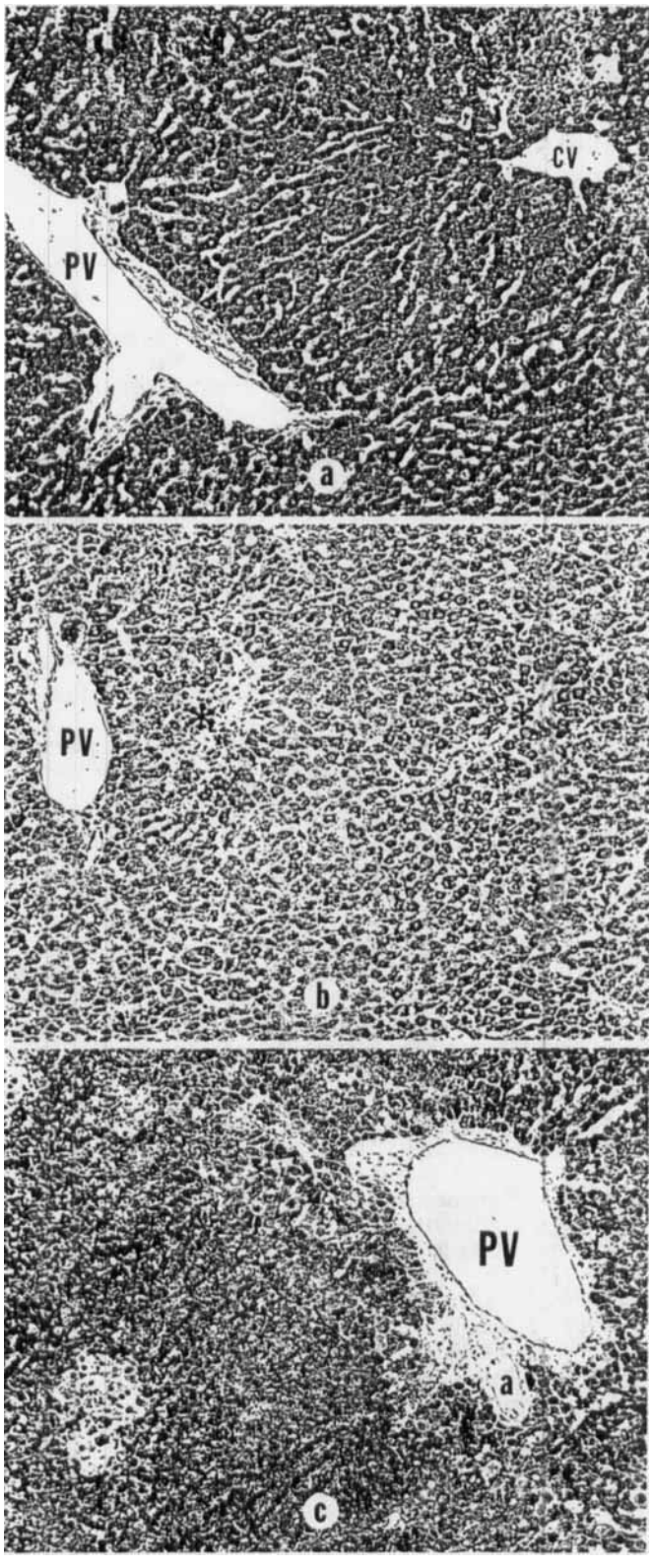

Fig. 2a–c. Grades of tissue damage in transplanted rat livers. **a** Mild (grade 1) damage. Two foci of necrosis *(asterisks)* are present near the central vein (*CV*; terminal hepatic vein). The parenchyma and the portal tract appear normal. *PV*, Portal vein. H & E, ×160. **b** Moderate (grade 2) damage. Several foci of ischemic necrosis *(asterisks)* are scattered throughout the lobule. The hepatocytes in the remaining parenchyma appear slightly dissociated and rounded up but viable. *PV*, Portal vein. H & E, ×73. **c** Severe (grade 3) damage. Extensive ischemic and hemorrhagic necrosis of hepatic parenchyma in middle and left third of picture. Parenchyma in the right third appears congested but viable. *PV*, Portal vein; *a*, hepatic artery. H & E, ×117

Aspartate aminotransferase (AST)

The median AST value, obtained by cardiac puncture during laparotomy of six normal rats, was 105 IU/l. Except for the median AST value in the UW group with 1 h of preservation, the AST values for all other groups were significantly elevated (Table 1). The AST values of the EC groups with 1, 12, and 24 h of preservation were all above those of the UW groups. For 30 h of preservation, comparison was not possible as all rats in the EC group died prematurely.

Histology

The severity of the histological changes increased with the duration of preservation in the EC as well as in the UW groups (Table 1; Fig. 2a–c). However, except for 1 h of preservation, the ischemic changes were consistently worse in the EC group. This difference between the EC and UW groups was already marked at 12 h of preservation. Although 11 of the 12 rats in the UW groups with 24 and 30 h of preservation survived up to 48 h post-transplantation, 10 of these rats showed severe ischemic changes in their livers.

Discussion

The liver transplant model in the rat with reconstruction of the hepatic artery is an excellent model for short and long-term survival studies [5, 11]. The low perioperative mortality (10%), the low AST levels, and the normal histology on the 2nd postoperative day in 75% of the experiments with 1 h of cold preservation indicate the stability of this model. Moreover, we achieved survival after 24 and 30 h of preservation, something which has not been published previously. We believe that the reconstruction of the hepatic artery is essential to obtain this stability of results and vitality of the graft.

Ontell et al. [10] have demonstrated the superiority of UW solution to EC solution in 12- and 24-h preservation studies in rats using an isolated perfusion model. They noted in UW-stored livers a significantly smaller increase in weight or even weight loss, less liberation of glucose and transaminases, and increased bile production. However, from our own experience with an in vitro perfusion model [4], the results cannot be extrapolated to a transplant model. In contrast, our study reflects the in vivo effect of EC and UW solutions on long-term liver preservation.

The marked difference in weight change in the liver between UW and EC solutions illustrates the effectiveness of lactobionate and raffinose in preventing intracellular edema as compared to glucose, which enters the cell freely. This supports the observation in isolated perfusion studies that lactobionate and raffinose cannot be omitted from UW solution without reducing its effectiveness [6]. The weight change in the liver seems to parallel the release of transaminases. This supports the concept that cellular swelling leads to cell damage, as demonstrated by elevated transaminase levels. The serum AST levels are

consistently higher in the EC groups than in the UW groups, irrespective of the duration of preservation.

In addition, the duration of preservation exerts its effect on transaminase levels, as the AST level on day 2 postoperatively increased with the duration of preservation in the EC as well as in the UW groups. Moreover, when the results of 1-h and 24-h preservation were compared, the AST level on day 2 postoperatively had increased twice as much in the EC group (tenfold) as in the UW group (fivefold). This illustrates the effectiveness of UW solution in the prevention of significant parenchymal damage up to 24 h. However, the sharp increase in AST levels with 30 h of preservation indicates that beyond 24 h of preservation, increased parenchymal damage is to be expected.

The same observations were made with histology on day 2 postoperatively. Except for the groups with 1 h of preservation, the EC groups showed persistently more ischemic damage than the UW groups. Also, wih increasing preservation times, the ischemic changes became more severe. At 30 h of preservation, none of the EC livers were life-sustaining in contrast to five out of six UW livers. However, all of these livers demonstrated moderate or severe ischemic damage.

The best proof of adequate preservation is the demonstration of life-sustaining function after transplantation. With EC solution, 50% of the animals in the group with 24 h of preservation and all of those in the group with 30 h of preservation died immediately post-transplantation. EC solution can, therefore, be considered inadequate beyond 12 h of preservation. This is supported by the findings that rat livers perfused with and stored in EC solution showed irreversible damage to the sinusoidal lining cells after 12 h, indicating that the safe period for storage is less than 12 h [9]. In contrast, with UW solution, all livers with 24 h of preservation and five of the six livers with 30 h of preservation were life-sustaining.

Thus, in our rat liver transplant model, UW solution proved to be superior to EC solution. However, the safety of UW solution for 24 h of preservation and beyond becomes questionable when one considers the levels of transaminases and the degree of ischemic changes at histology.

Although clinically liver preservation up to 24 h has been reported with UW solution [8], preservation beyond 15 h [12] appears to markedly increase transaminase levels (above 1000 IU/l) in one-third of the patients and leads to a severely abnormal prothrombin time (> 20 s) in half of the patients. Although no relationship was found between the duration of preservation and primary graft failure, the above-mentioned abnormalities in clinical transplantation and the findings in our rat study suggest caution in extending liver preservation with UW solution beyond 24 h.

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