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Rat liver preservation

II. Combining UW solution with Eurocollins solution or Ringer's lactate abrogates its protective effect

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Abstract. The mechanisms by which cold preservation solutions exert their protective effects are only partially understood. The consequences of mixing different solutions, with presumably different modes of action, may be additive and beneficial or may be deleterious. It is commonplace in clinical liver preservation to use Ringer's lactate (RL), Eurocollins (EC), and University of Wisconsin (UW) solution in sequence for washout of blood, precooling, and cold storage of the organ. In this study, 114 Sprague Dawley rats received orthotopic liver transplants that were flushed in various sequences with RL, EC, and UW solutions. One-week animal survival served as the criterion of preservation success. The results demonstrated that liver preservation with UW solution alone is significantly superior ($P < 0.01$) to any combination of RL, EC, and UW solutions and may explain some of the instances of primary nonfunction in clinical liver transplantation.

Key words: UW solution in liver preservation, in the rat – Liver preservation, experimental, rat – Preservation, liver, UW and other solutions – Washout liver, experimental, rat

We recently had unexpected postoperative malfunction in two otherwise uneventful liver transplants that had been cold-stored for 10 and 11 h, respectively, in University of Wisconsin (UW) solution [4]. In both instances donor hepatectomy in other centres was preceded by in situ washout with cold Eurocollins (EC) solution [2], and only after excision and prior to storage was EC replaced by UW. The discouraging outcome in our two patients receiving livers not exclusively preserved by UW is in keeping with observations by Belzer [1] and led us to a re-evaluation of Starzl's precooling strategy in liver procurement [8, 11]. We hypothesized that such mixing of preservation fluids may prejudice postoperative function in liver transplantation. Therefore, we designed a series of experiments to mimic practice in the clinic in which during dissection an initial flush, often Ringer's lactate (RL), is used to clear blood from the liver, followed by a continuous flush of preservation solution after the circulation has been clamped and during removal of the liver. A third flush is commonly employed after the liver has been removed in preparation for cold storage and transplantation. Finally, a flush at the time of implantation clears the cold storage solution before the circulation is re-established. We report here a series of 114 rat liver transplants in which we have tested various washout combinations.

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Materials and methods

Animals and techniques

The transplants between partially inbred donor and recipient Sprague Dawley rats were performed using a modified Kamada technique [5] described by us recently [12]. The donor livers were skeletonized and then cold-flushed in situ through the portal vein. Three sequential flushes of 10 ml aliquots of test solutions (4°C) were achieved by gravity from a height of 20 cm as follows. Immediately after washout with the first solution, the second solution was flushed through the liver and remained for 30 min to mimic the period of final dissection in humans before being replaced by the third, or storage, solution in which the liver was left for 8 h and 20 min on melting ice at 1°C. Finally, in all livers, the storage solution was flushed out with cold RL just before implantation, which usually took about 10 min. Thus, the total ischemic time was 9 h. A total of

120 animals received livers but 6 were excluded as technical failures, leaving 114 with satisfactory grafts. Postoperatively, all animals were examined and weighed daily, and those that were ill or dying were sacrificed; this was counted as the day of death. All remaining animals were sacrificed in good health at 1 week. All animals had post mortems. Vascular anastomoses were inspected and liver samples were taken for H & E morphology.

Experimental groups

The animals were divided in seven groups according to the types and sequence of solutions used (Fig. 1):
Group 1 ($n = 18$): UW solution for all three stages; initial flush, in situ cooling and cold storage
Group 2 ($n = 22$): Initial flush with RL, next two stages with UW solution
Group 3 ($n = 11$): Just first two stages with RL, cold storage in UW solution
Group 4 ($n = 9$): EC solution as initial flush, followed by UW solution for stages two and three
Group 5 ($n = 15$): EC solution for stages one and two, and UW solution for storage
Group 6 ($n = 19$): RL as initial flush, in situ cooling with EC solution and storage in UW solution
Group 7 ($n = 20$): RL as initial flush, EC solution for next two stages
In all seven groups the preanastomotic final flush was with cold RL.

Results

The results are summarized in Fig. 1. Only group 1, in which UW solution was used throughout, achieved acceptable survival. Group 6, which mimics the common clinical situation, had only 47% survival.

All animals that died, died of liver failure 1–3 days after transplant. Histologically, the usual pattern of infarction, coagulation necrosis, and extensive liver cell dropout was seen. This differed distinctly from those animals sacrificed in good health at 1 week, in which histologically normal liver architecture and minimal or absent cellular damage was seen.

Discussion

In our laboratory rat liver preservation model, an initial flush with RL, followed by in situ cooling and cold storage in UW solution, has been our standard against which experimental solutions are compared. The 9-h time period was chosen because it is the point at which approximately 50% of the animals survive and because it parallels the clinical situation. Shorter preservation times result in universal survival in this rat model [12].

The results in this paper were puzzling but not unexpected. That an initial flush with cold RL might prejudice liver graft survival has already been suggested by Otto et al. [7], who showed in pigs that Ringer's lactate at 4°C induced severe sinusoidal endothelial damage, which seems to be the critical injury in preservation-damaged liver grafts [6] and apparently is minimal if UW solution is used at all stages [3]. Recently, Sundberg et al. reported similar findings in a rabbit liver perfusion

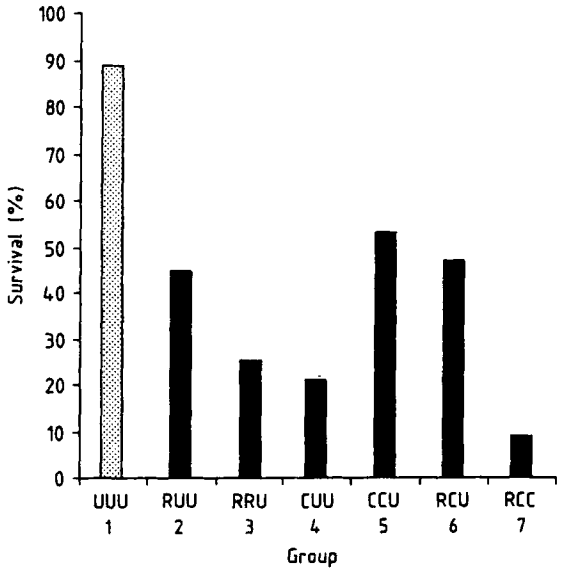

Fig. 1. One-week rat survival after orthotopic liver transplantation with 9-h preservation time. Livers were flushed through portal vein for 1 min with 10 ml ice cold compound no. 1 *(first letter under bars)*, followed by 10 ml compound no. 2 *(second letter under bars)*, and after 30 min with 10 ml compound no. 3 and storage on ice for 8.5 h. All results are statistically significant when compared to group 1, UUU, using Fischer's two-tailed exact test with P values < 0.05 being considered significant. *U*, UW solution; *R*, Ringer's lactate; *C*, Eurocollins solution. Group 1: $n = 18$; group 2: $P < 0.01$, $n = 22$; group 3: $P < 0.01$, $n = 11$; group 4: $P < 0.01$, $n = 9$; group 5: $P < 0.03$, $n = 15$; group 6: $P < 0.01$, $n = 19$; group 7: $P < 0.01$, $n = 20$

model in which in vitro bile production was the marker rather than survival [9]. In summary, our results suggest that:

1. A brief exposure of rat livers prior to cold storage to either RL (group 2), EC solution (group 4), or both (group 6) is deleterious to the protective effect that UW solution affords and reduces the latter from an extraordinary to an ordinary preservation fluid.

2. The quality of RL or EC-induced injury, because it is set so swiftly, cannot conceivably be irreversible and of ischemic nature in a classical sense, e.g., cell swelling, membrane breakdown, energy storage-supply failures, etc. This is supported by the close to 100% survival of animals whose livers were washed out as described but reimplanted immediately (data not shown).

3. The quality of injury may rather be thought of in terms of subtle, molecular, cell membrane-associated changes that later on, during cold storage, inhibit the cold protection mechanism(s) of UW solution.

4. The best preservation solution now available for liver preservation, namely UW solution, should be used from first to last, and attempts to save money by using the much cheaper substitutes may prove costly.

It remains to be seen whether, in this model, changing the final flush solution to something other than RL might further improve the results, as recently suggested by Wood et al. [10].

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