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Warm Carolina rinse solution prevents graft failure from storage injury after orthotopic rat liver transplantation with arterialization

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Abstract. An injury to nonparenchymal cells, characterized by loss of viability of sinusoidal endothelial cells and activation of Kupffer cells, occurs after reperfusion of livers stored for transplantation. Recently, a new solution, Carolina rinse solution, was shown to prevent reperfusion injury to endothelial cells in vitro almost completely and to improve graft survival after orthotopic rat liver transplantation (ORLT) without arterialization. ORLT with arterialization permits longer cold storage of donor livers and more closely models human surgery. Therefore, we evaluated the effects of Carolina rinse solution on graft survival after ORLT with arterialization in syngeneic Lewis rats. Just prior to implantation, donor livers stored in University of Wisconsin (UW) solution were rinsed with 30 ml of Ringer's solution, saline, or Carolina rinse solution at 1°–4°C. In livers stored for 15 h and rinsed with Ringer's or saline solution, long-term graft survival was only 8%. Using Carolina rinse solution containing 1 mmol and 200 μmol adenosine per liter, graft survival improved to 40% and 80%, respectively. Graft survival did not improve when using Carolina rinse solution with adenosine omitted or Ringer's solution containing 200 μmol adenosine per liter. Livers were also rinsed with Carolina rinse solution containing 200 μmol adenosine per liter at 28°–30°C rather than at 1°–4°C. With warm Carolina rinse solution, survival improved further to 100%, 80%, and 50% after 15, 18, and 21 h of storage. After 18 h of storage, light and electron microscopy demonstrated marked denudation of the sinusoidal lining and activation of Kupffer cells in grafts rinsed with Ringer's solution. Use of Carolina rinse solution greatly improved endothelial structure but did not reduce Kupffer cell activation. In conclusion, these findings show that Carolina rinse solution substantially improves graft survival after ORLT with arterialization. Adenosine and warm temperature are important factors contributing to efficacy. A mechanism of protection appears to be prevention of reperfusion-induced endothelial cell injury.

Key words: Rat liver transplantation, Carolina rinse solution – Liver transplantation, rat, Carolina rinse solution – Preservation, rat, liver

In liver grafts that will fail as a consequence of storage injury, a reperfusion injury to nonparenchymal cells occurs that is characterized by loss of viability of sinusoidal endothelial cells [4, 5]. By contrast, hepatocytes, Kupffer cells, fat-storing cells, and intra-hepatic bile duct epithelial cells retain viability. In addition, Kupffer cells become activated after reperfusion of stored livers, as evidenced by structural changes (ruffling, degranulation, etc.), release of lysosomal enzymes, formation of oxygen-free radicals, and increased phagocytosis [5, 6, 8, 21, 29]. A reperfusion injury also occurs in vivo after orthotopic rat liver transplantation (ORLT) [32], and both endothelial cell killing and Kupffer cell activation can be documented by electron microscopy postoperatively in failing liver grafts [7, 33].

Recently, we described a new solution – Carolina rinse solution – designed to prevent reperfusion injury to livers stored for transplantation [9]. Containing antioxidants, vasodilators, substrates for ATP regeneration, high molecular weight osmotic support, electrolytes similar to serum, and mildly acidic pH, Carolina rinse solution almost completely prevented reperfusion-induced killing of endothelial cells in stored rat livers. In addition, Carolina rinse solution prevented lethal injury in suspensions of ATP-depleted hepatocytes. It was particularly effective in comparison to University of Wisconsin (UW) solution at warm temperatures (23°–37°C) [10]. Most significantly, rinsing of liver grafts with cold Carolina rinse solution just prior to implantation markedly improved graft survival after ORLT without rearterialization [13]. Adenosine accounted in large part for the efficacy of Carolina rinse solution in vivo [12, 13].

Recently, a new cuff technique was developed for arterialization of implanted rat liver grafts [24]. In comparison

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to livers transplanted without arterialization, grafts transplanted using this new technique survived after longer storage times [25]. Transplantation with arterialization resembles clinical transplantation more closely and presumably permits improved perfusion of implanted grafts. Accordingly, one aim of this study was to determine whether Carolina rinse solution would also improve survival after ORLT with arterialization. A second aim of this study was to compare the efficacy of cold and warm Carolina rinse solution in preventing graft failure, since cytoprotection by Carolina rinse solution was temperature-dependent in vitro and since graft survival in vivo also improved when grafts were rinsed with warm compared to cold Ringer's solution [30]. A third aim was to evaluate the importance of adenosine in the efficacy of Carolina rinse solution for arterialized grafts.

Materials and methods

Surgery

Livers of syngeneic male Lewis rats (250–300 g) were transplanted under ether anesthesia essentially as described by Steffen et al. [24]. In the donor operation, the donor liver was flushed via the portal vein with chilled UW solution. The superior vena cava, inferior vena cava, portal vein, celiac artery near the aorta, and bile duct were divided and the liver was excised. Cuffs were placed on the portal vein and inferior vena cava, and the liver was stored in UW solution in an ice-water bath for up to 24 h. In recipient rats, the hepatic and gastroduodenal arteries were divided between ligatures at their origin, leaving a stump of the common hepatic artery. The stump was clamped at the base of the dissected segment and cut at the bifurcation of the hepatic and gastroduodenal arteries. This procedure left a funnel-shaped opening to which a cuff was attached. After dividing the bile duct at the hilum, the superior vena cava, inferior vena cava, and portal vein were clamped and divided and the recipient liver was removed. The donor liver was then rinsed with 30 ml of saline solution, Ringer's solution, or various experimental solutions (Table 1) and placed in the abdomen. The temperature of the rinse solutions was 1°–4°C or 28°–30°C, as specified. Subsequently, the superior vena cava was anastomosed with a running suture and the portal vein, inferior vena cava, and hepatic artery were connected in sequence by insertion of cuffs. The bile duct was anastomosed over an intraluminal polyethylene splint. Implantation surgery required 60 min. During the time, the portal vein was clamped for 15 min and the inferior vena cava for not more than 20 min. Rats were given food and water ad libitum postoperatively.

Measures of survival

Both long-term survival and average days of survival (with 30 days as a maximum) were used as indices of experimental outcome, as described previously [12, 13, 30, 33]. Long-term survival was defined as the percent or fraction of rats living 30 days postoperatively, a time after which indefinite survival was virtually assured. Average days of survival, in contrast to percent long-term survival, provided a discriminating measure of outcome for treatments in which animals did not survive 30 days. After 30 days, all transplant recipients were sacrificed.

Histology

For light and electron microscopy, recipient rats were anesthetized with pentobarbital 2 h postoperatively. A cannula was inserted into the portal vein and Krebs-Henseleit bicarbonate buffer saturated with 95% oxygen, 5% carbon dioxide at 37°C was infused for 2 min at 15 ml/min. The intrahepatic vena cava was cut to allow drainage. Subsequently, warm fixative containing 2% glutaraldehyde, 2% formaldehyde in 0.1 mol NaPi per liter buffer, pH 7.4 was infused for another 2 min. The livers were then excised and prepared for light microscopy and scanning electron microscopy as previously described [4, 5, 8]. Plastic sections (2–4 μm) for light microscopy were stained with methylene blue and acid fuchsin.

Statistics

Average survival times were compared using one-tailed *t*-tests. Long-term survival was compared by one-tailed Fischer's exact tests. Statistical significance was taken as a *P* value of 0.05 or less. Probability values between 0.05 and 0.10 ("marginal significance") were also noted.

Materials

Nicardipine and modified hydroxyethyl starch (pentafraction) were the gifts of DuPont (Wilmington, Del., USA). Other reagents were obtained from standard commercial sources.

Results

Graft survival after ORLT with arterialization

For livers stored for 12 h in UW solution and rinsed with either saline solution or Ringer's solution at 1°–4°C, 21 out of 22 recipient animals survived 30 days (Table 2, Fig. 1). For livers stored 15 h or longer, only 1 of 14 animals in the saline group and 1 of 20 animals in the Ringer's group survived 30 days. Typically under nonsurvival conditions, animals awoke normally from surgery, ambulated, and drank water. After 4–10 h, however, their condition began to deteriorate. Prior to death the animals became unresponsive to external stimuli and breathed with difficulty. At necropsy, the lungs were spotty and hemorrhagic, and the pleural cavities contained 1–2 ml of serous effusion. The liver, bowel, and kidneys were dark red. No evidence

Table 1. Composition of rinse solutions in units per liter. Solution A is Carolina rinse solution is originally described [15]. Solutions B and C are modifications of Carolina rinse solution in which adenosine content is altered. Solution D is Ringer's solution containing adenosine. HEPES, 4-[2-hydroxyethyl]-piperazine ethanesulfonic acid; HES, hydroxyethyl-starch; MOPS, 3-[N-morpholino]propanesulfonic acid

Component	Saline	Ringer's	A	B	C	D
NaCl (mmol)	145	115	115	115	115	115
KCl (mmol)	–	5	5	5	5	5
$CaCl_2$ (mmol)	–	2	1.3	1.3	1.3	2
KH_2PO_4 (mmol)	–	1	1	1	1	1
$MgSO_4$ (mmol)	–	1.2	1.2	1.2	1.2	1.2
NaHEPES (mmol)	–	25	–	–	–	25
HES (g/l)	–	–	50	50	50	–
Allopurinol (mmol)	–	–	1	1	1	–
Desferrioxamine (mmol)	–	–	1	1	1	–
Glutathione (mmol)	–	–	3	3	3	–
Nicardipine (μmol)	–	–	2	2	2	–
Adenosine (mmol)	–	–	1	0.2	0	0.2
Fructose (mmol)	–	–	10	10	10	–
Glucose (mmol)	–	–	10	10	10	–
Insulin (IU/l)	–	–	100	100	100	–
MOPS (mmol)	–	–	20	20	20	–
pH	[a]	7.4	6.5	6.5	6.5	7.4

[a] Unbuffered

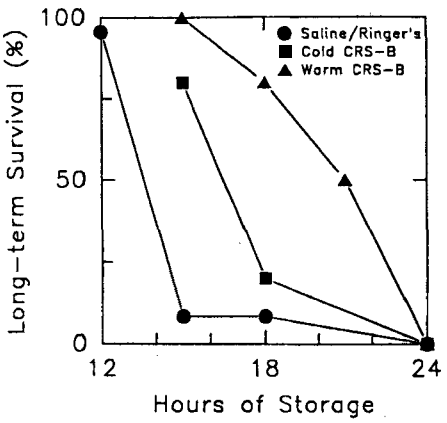

Fig. 1. Long-term survival (> 30 days) of liver grafts after treatment with Ringer's solution and Carolina rinse solution. Rat livers were stored in UW solution and transplanted after rinsing with cold Ringer's solution (●), cold solution B (■), or warm solution B (▲)

of infection was observed and none of the animals became obviously jaundiced. Since long-term survival in the saline and Ringer's groups was virtually identical (9/10, 0/2, 1/7, and 0/5 for saline and 12/12, 1/10, 0/5, and 0/5 for Ringer's solution, respectively, after 12, 15, 18, and 24 h of storage), the data for the saline and Ringer's groups were combined in Table 2 for comparison with Carolina rinse solution and its variants. For conciseness, the latter solutions are named solutions A, B, C, and D (Table 1).

Increased survival of arterialized liver grafts rinsed with Carolina rinse solution

For livers stored for 15 h and rinsed with cold Carolina rinse solution (solution A) prior to implantation, 2 of 5 grafts survived 30 days, as compared to 1 long-term survival out of 12 operations for grafts rinsed with saline or Ringer's solution (Table 2). Average survival time improved to 13.3 days after solution A from 3.5 days with saline/Ringer's solution, a statistically significant increase. However, survival after 18 h of storage did not improve.

Role of adenosine in improved graft survival after ORLT with arterialization

Adenosine is a component of both UW solution and Carolina rinse solution. For UW solution, adenosine is considered a key ingredient contributing to improved preservation during storage [3]. Similarly, adenosine has been found to be an essential component of Carolina rinse solution, responsible for improving survival of nonarterialized grafts [12, 13]. Adenosine is more effective at low than at high concentrations [12], possibly because wash-in of adenosine from organ implants can cause cardiac arrhythmias [23]. Accordingly, we evaluated Carolina rinse solution containing 200 μmol adenosine per liter (solution B) rather than 1 mmol. With solution B, four out of five animals survived 30 days after 15 h of storage, a statistically significant improvement over the saline/Ringer's solution group (Table 2, Fig. 1). Average survival time with solution B was 24.2 days, seven times the survival time of the combined saline and Ringer's solution groups (Table 2). After 18 and 24 h of storage, 30-day survival of livers flushed with solution B was one out of five and zero out of two, respectively. However, average survival times remained greater in grafts treated with solution B than with saline and Ringer's solutions (Table 2).

Another study was performed in which adenosine was eliminated entirely from Carolina rinse solution (solution C). With solution C, 30-day survival decreased to zero out of five after 15 h of storage, and average survival time declined to 1.4 days, not a significant improvement over the combined saline and Ringer's solution groups (Table 2). To determine whether the efficacy of Carolina rinse solution was due to adenosine alone, liver grafts were also rinsed with Ringer's solution containing 200 μmol adenosine per liter (solution D) prior to implantation. After 15 h of storage and rinsing with solution D, 30-day survival was only one out of five, and average survival time was not significantly greater than the corresponding saline/Ringer's group (Table 2). Two livers were also flushed with solution D after 18 h of storage, but neither survived.

Effect of temperature on protection by Carolina rinse solution

In a recent study, the relative protection by Carolina rinse solution against loss of cell viability in ATP-depleted hepatocytes was greatest at temperatures between 23° and 37°C in vitro [10]. Moreover, when Ringer's solution was used as a rinse solution in nonarterialized grafts, warm temperature of the rinse improved survival [30]. Therefore, we investigated the role of temperature on the efficacy of Carolina rinse solution containing 200 μmol adenosine per liter (solution B) in preventing graft failure from storage injury. In grafts stored for 18 h and rinsed with warm solution B, long-term survival was four out of five animals, a marginally significant ($P = 0.10$) increase

Table 2. Graft survival after orthotopic rat liver transplantation with rearterialization following cold rinses with various solutions. CRS, Carolina rinse solution

Rinse solution	Storage time	Survival days ± SD	30-day survivors/ Total transplants
Saline/Ringer's solution	12 h	28.7 ± 6.2	21/22
	15 h	3.5 ± 8.4	1/12
	18 h	3.1 ± 8.5	1/12
	24 h	0.4 ± 0.1	0/10
Solution A (CRS, 1 mmol adenosine)	15 h	13.3 ± 15.3*	2/5
	18 h	1.3 ± 1.5	0/6
Solution B (CRS, 200 μmol adenosine)	15 h	24.2 ± 12.9***	4/5**
	18 h	8.3 ± 12.3	1/5
	24 h	0.8 ± 0.4**	0/2
Solution C (CRS, no adenosine)	15 h	1.4 ± 1.9	0/5
Solution D (Ringer's solution, 200 μmol adenosine)	15 h	7.2 ± 12.7	1/5
	18 h	0.8 ± 0.1	0/2

* $P < 0.05$ compared to saline/Ringer's solution; ** $P < 0.01$; *** $P < 0.001$

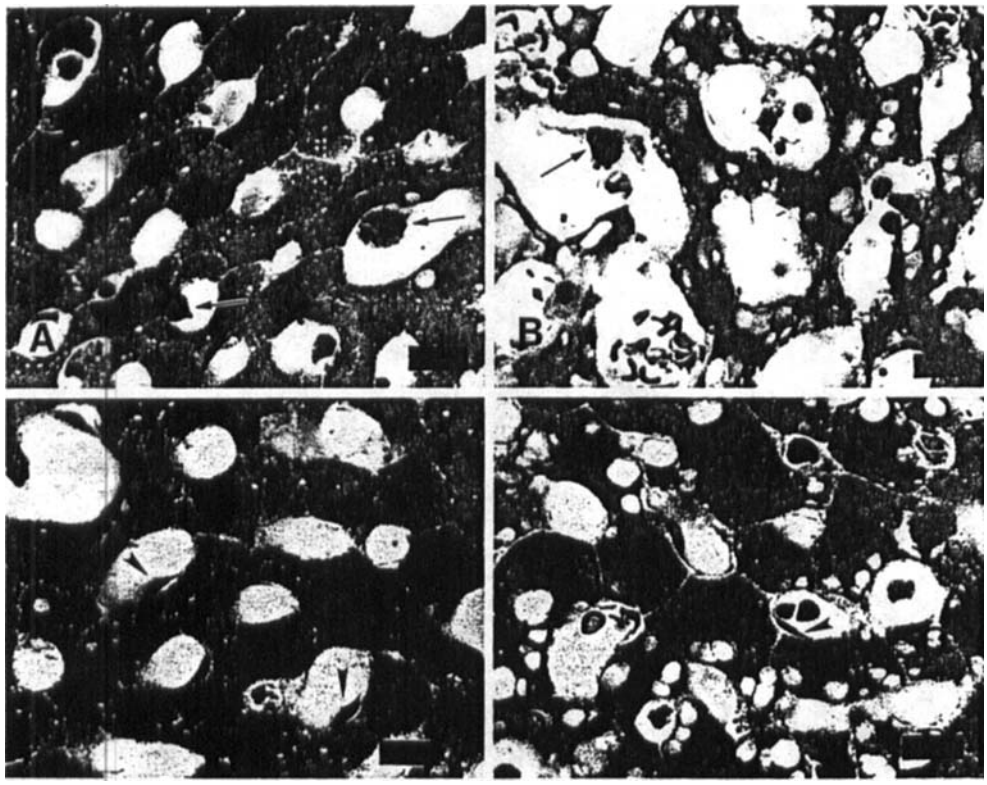

Fig. 2 A–D. Light microscopy of transplanted rat livers after treatment with Ringer's solution or Carolina rinse solution. In livers treated with Ringer's solution, periportal regions (**A**) exhibited abnormally rounded nonparenchymal cells *(arrows)* with normal parenchymal cells. In pericentral regions (**B**), parenchymal cell vacuolization was usually evident together with a more marked disturbance of nonparenchymal cells. In livers treated with solution B, both periportal (**C**) and pericentral (**D**) regions displayed flattened sinusoidal lining cells *(arrowheads)*, which adhered closely to the underlying parenchymal cells. Pericentral but not periportal parenchymal cells were again vacuolated. *Bar:* 20 μm

over cold solution B and a highly significant ($P < 0.001$) increase over the saline/Ringer's group. Average survival time was also significantly increased in comparison to cold solution B and the saline/Ringer's groups (Table 3, Fig. 1). After 21 h of storage, long-term survival was still 50% with warm solution B (Table 3, Fig. 1). After 24 h of storage, zero out of five animals survived 30 days after warm solution B, but average survival times remained higher, a trend that was marginally significant ($P = 0.10$). In comparison to the saline/Ringer's solution group, the improvement of survival time by warm solution B was significant ($P < 0.01$) at all storage times examined (Table 3).

Structural changes postoperatively in grafts treated with Ringer's solution and warm Carolina rinse solution

Carolina rinse solution containing 200 μmol adenosine per liter (solution B) used at 28°–30°C was the most effective in preventing liver graft failure. To determine the structural features associated with graft success and failure, livers were stored for 18 h, transplanted, and fixed 2 h postoperatively after treatment with Ringer's solution or warm solution B. In liver grafts rinsed with Ringer's solution where long-term survival was less than 10%, light microscopy revealed intact hepatic parenchymal cells with vacuolization in pericentral areas, but little hepatocellular necrosis (Fig. 2 A, B). By contrast, nonparenchymal structures were severely disrupted. In many regions, cells lining the sinusoids were absent. Elsewhere, necrotic nonparenchymal cells containing pyknotic nuclei projected into sinusoidal lumens. By contrast, in grafts rinsed with solution B where long-term survival was 80%, the integrity of the sinusoidal lining was greatly improved, although pericentral parenchymal cells remained vacuolated (Fig. 2 C, D). In most other respects, grafts treated with warm solution B were judged normal by light microscopy 2 h postoperatively.

Grafts were also examined by scanning electron microscopy. Livers transplanted under nonsurvival conditions using Ringer's rinse showed extensive areas of nearly complete endothelial denudation (Fig. 3 A, B). Elsewhere, fenestrations of the endothelium were greatly enlarged, and endothelial cell cytoplasm was drawn back into relatively thick cords, as described previously for liver reperfused in vitro [8]. In addition, Kupffer cells in both periportal and pericentral regions were swollen with their surfaces covered with blebs and ruffles. The extent of ruffling was greater in pericentral regions where endothelial injury was also more marked. The appearance of hepatic parenchymal cells, however, was nearly normal. Biliary,

Table 3. Graft survival after orthotopic rat liver transplantation with rearterialization with warm Carolina rinse solution containing 200 μmol/l adenosine (solution B) at 28°–30°C

Storage time	Survival days ± SD	30-day survivors/ Total transplants
15 h	30 ± 0[a]	2/2[b]
18 h	24.2 ± 12.9[c]	4/5[d]
21 h	16.5 ± 15.6	2/4
24 h	1.8 ± 0.9[e]	0/5

[a] $P < 0.001$ compared to saline/Ringer's solution (Table 2)
[b] $P < 0.05$ compared to saline/Ringer's solution (Table 2)
[c] $P < 0.05$ compared to 1°–4°C (Table 2); $P < 0.001$ compared to saline/Ringer's solution (Table 2)
[d] $P = 0.10$ compared to 1°–4°C (Table 2); $P < 0.01$ compared to saline/Ringer's solution (Table 2)
[e] $P < 0.001$ compared to saline/Ringer's solution (Table 2)

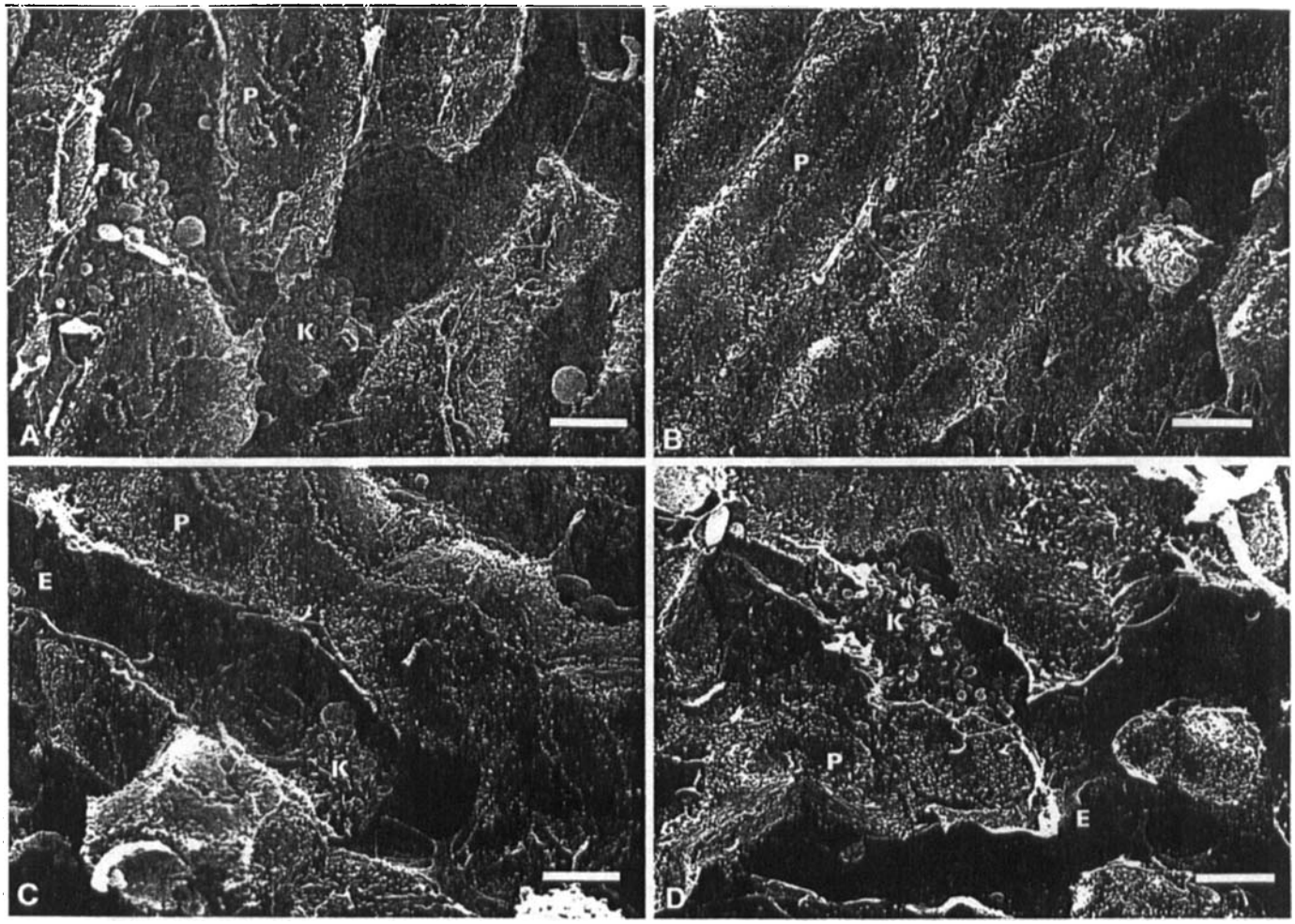

Fig. 3 A–D. Scanning electron micrographs of transplanted rat livers after treatment with Ringer's solution or Carolina rinse solution. In livers rinsed with Ringer's solution, periportal areas (**A**) exhibited marked disruption of the sinusoidal endothelium. Pericentral regions (**B**) were virtually denuded of endothelium. Kupffer cells *(K)* exhibited rounding and ruffling characteristic of macrophage activation. Parenchymal cells *(P)* retained a normal surface appearance in both periportal and pericentral regions. After treatment with Carolina rinse solution (solution B), the sinusoidal endothelium *(E)* was relatively undisturbed in both periportal (**C**) and pericentral (**D**) regions. By contrast, Kupffer cells were rounded and ruffled to about the same extent as in livers treated with Ringer's solution. The surface structure of parenchymal cells was normal. *Bar:* 5 μm

intercellular, and sinusoidal surfaces all displayed structural detail typical of control livers [8, 20].

In liver grafts treated with solution B, injury to the sinusoidal lining was diminished greatly (Fig. 3 C, D). Areas of endothelial denudation were uncommon. In most areas, endothelial fenestrations were enlarged, but the extent of change was less than after treatment with Ringer's solution. However, Kupffer cells remained swollen, blebbed, and ruffled. These structural changes, indicating macrophage activation, appeared to be nearly as great in grafts treated with solution B as in failing grafts treated with Ringer's solution.

Discussion

Efficacy of Carolina rinse solution

Previously, we developed a new solution – Carolina rinse soluton – that prevented lethal reperfusion injury after storage almost completely in isolated perfused rat livers and that improved survival of nonarterialized grafts [9, 12, 13]. Here, we show that rinsing with Carolina rinse solution after storage, just prior to implantation, also prevents graft failure after orthotopic rat liver transplantation with arterialization. As a consequence, storage times in this model were extended from a maximum of 12 h in livers rinsed with Ringer's solution to nearly 21 h in livers rinsed with Carolina rinse solution (solution B). A significant improvement in survival was observed both in the proportion of long-term (30-day) survivors and in the average days of survival postoperatively.

Role of adenosine

Adenosine was an important component contributing to the efficacy of Carolina rinse solution for arterialized liver grafts. When adenosine was omitted from Carolina rinse solution, efficacy was lost. However, adenosine alone in Ringer's solution did not improve graft survival. Thus, other components of Carolina rinse solution must be acting synergistically with adenosine to produce the beneficial effect. Carolina rinse solution shares many ingre-

dients with UW solution. However, it acts differently from UW solution since in previous experiments with ORLT without arterialization, rinsing with UW solution prior to implantation was no better for survival than rinsing with Ringer's solution [14]. Identification of the other key components of Carolina rinse solution awaits future study.

Adenosine may act by way of a number of mechanisms. By stimulation of purinergic receptors, adenosine depresses phagocytosis by macrophages [11] and causes hepatic vasodilation by relaxation of vascular smooth muscle cells [19]. Adenosine is also a substrate for regeneration of adenine nucleotides depleted during cold storage [2, 22]. This effect probably does not account for the beneficial effect of adenosine on graft survival since adenosine was effective at 200 μmol per liter, whereas millimolar concentrations would be required to regenerate ATP to normal cellular levels. Indeed, higher concentrations of adenosine (1 mmol/l) caused a poorer postoperative outcome, in agreement with experiments employing nonarterialized grafts [12]. Moreover, the metabolic precursors adenine and ribose cannot replace adenosine to improve survival [12]. These findings are also consistent with deleterious systemic effects of high adenosine on cardiovascular function observed clinically [23].

In confirmation of the recent report by Steffen et al. [25], ORLT with arterialization was successful after longer periods of liver storage than ORLT without arterialization. For example, long-term survival after 12 h of storage in UW solution was almost 100% in arterialized grafts (Table 2), but in our hands it was 0% without arterialization after only 8 h of storage [29]. Carolina rinse solution also improved graft survival in a model of rat liver transplantation without arterialization, an effect linked to adenosine [12, 13]. In the nonarterialized model, adenosine alone was effective in improving long-term graft survival. This contrasts with the present findings using arterialized grafts. Reasons for this difference are unclear. However, in nonarterialized grafts, blood perfusion may be suboptimal, and microcirculatory deficits develop, especially in grafts that are failing from storage injury [31]. Adenosine is a potent vasodilator that may account for the efficacy of adenosine alone in the nonarterialized model.

Length of safe storage of untreated livers

Recently, long-term survival rates of 29%–100% after 24 h of storage in UW solution have been described following rat liver transplantation [1, 15, 26, 28, 29, 34, 36]. In the present study, we could not obtain long-term survival after storage for more than 15 h without using Carolina rinse solution. Since the success of our surgery after 12 h or less of storage was extremely high – approaching 100% – factors related to donor and recipient size, nutritional status, and exact temperature of storage may be responsible for this difference between our study and those of others. For example, we stored our livers in an ice-water bath and monitored temperature to assure that it was in the expected 0°–1°C range. Many others performing rat liver transplantations store liver explants in refrigerators at about 4°C. Recent clinical and experimental reports on livers and other organs suggest that such warmer temperatures improve organ preservation [16–18, 35]. In addition, survival rate is highly dependent upon the rat strain employed [27]. Thus, these or other factors may explain the relatively shorter safe storage times that we observed in untreated livers.

Improvement by warm temperature

Warm (28°–30°C) Carolina rinse solution was substantially more effective in improving graft survival than cold (1°–4°C) rinse solution. This observation is consistent with previous findings in suspensions of ATP-depleted hepatocytes, which showed that relative protection by Carolina rinse solution, in comparison to UW and Ringer's solutions, is greatest at warm rather than at cold temperatures [10]. Warm temperature also improves the efficacy of Ringer's solution as a rinse solution, as demonstrated both by improved survival after ORLT without arterialization and by improved viability of ATP-depleted hepatocytes relative to UW solution [10, 30].

Mechanisms underlying protection

Previous work suggests that graft failure from storage-related reperfusion injury occurs by one or both of two mechanisms [4–8, 21, 29, 32]. The first mechanism is reperfusion-induced loss of viability of sinusoidal endothelial cells, which in vivo leads to platelet and leukocyte margination, hemostasis, inflammation, ischemia, and, ultimately, graft failure. The second mechanism is activation of Kupffer cells leading to the release of cytokines, oxygen radicals, and possibly other toxic mediators. As a result, a form of toxic shock develops, culminating in adult respiratory distress syndrome, multiple organ failure, and death of the host animal. In the present study, we confirm that failure of arterialized grafts from reperfusion injury associated with organ storage is linked both with the destruction of sinusoidal endothelial cells and with the activation of hepatic macrophages. Under conditions where warm Carolina rinse solution prevented graft failure from preservation injury, endothelial integrity was markedly improved, but Kupffer cell activation was not diminished. These observations suggest, therefore, that Carolina rinse solution improves graft survival by preventing reperfusion-induced killing of endothelial cells.

Whether the critical injury leading to graft failure is, in fact, a reperfusion injury has been a controversial issue [14]. The efficacy of Carolina rinse solution in vivo and in vitro in preventing graft failure and lethal injury to endothelial cells provides compelling evidence that the critical injury causing graft failure from preservation injury is, indeed, a reperfusion phenomenon. In conclusion, use of Carolina rinse solution to rinse rat liver grafts prior to implantation substantially improves postoperative survival. This simple strategy may be of value in a clinical setting to reduce the incidence of primary graft nonfunction and to improve hepatic function postoperatively.

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