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The second generation of Carolina Rinse, solution II, improves graft survival following orthotopic liver transplantation in the rat by preventing reperfusion injury

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Abstract. Carolina Rinse solution was designed to minimize reperfusion injury following orthotopic liver transplantation. Carolina Rinse blocks reperfusion-induced endothelial cell killing, diminishes postoperative enzyme release and improves survival dramatically. Adenosine and mildly acidotic pH were identified as key components. Here we report results with a simplified formulation, Carolina Rinse II, which contains extracellular inorganic ions similar to Ringer's solution, adenosine, as well as antioxidants and radical scavengers (allopurinol, glutathione and desferrioxamine). In this study, 44 rat livers were explanted and stored for 12 h in University of Wisconsin (UW) cold storage solution (non-survival conditions). Control livers were rinsed with 15 ml cold Ringer's solution just prior to completion of implantation surgery. In this control group, average 30-day survival was poor (8%). However, survival was increased to around 60% when grafts were rinsed with Carolina Rinse II. Survival was not improved significantly by rinsing the graft with Ringer's solution containing antioxidants and radical scavengers with adenosine omitted (about 30%). Peak SGOT values of nearly 3000 U/l, measured 1–3 days postoperatively in the Ringer's rinse control group, were decreased 4- to 5-fold both by Carolina Rinse II and by Ringer's solution containing antioxidants. On the other hand, the addition of adenosine to Ringer's solution improved survival (around 60%) but did not decrease the postoperative elevation of serum enzymes significantly. Thus, it appears that adenosine was necessary for optimal survival whereas antioxidants and radical scavengers were needed to prevent injury to the transplanted graft. These data were consistent with the hypothesis that at least two mechanisms, one involving the liver and a second one non-hepatic, are responsible for post-transplant pathophysiology. Carolina Rinse II also reduced the postoperative elevation in serum enzymes 2- to 3-fold in livers stored under survival conditions (e.g., for 8 h in UW solution). This study demonstrated convincingly that a very simple rinse solution, Carolina Rinse II, improved survival significantly and minimized graft injury following orthotopic liver transplantation.

Key words: Carolina Rinse II – Orthotopic liver transplantation – Graft survival – Reperfusion injury

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It has been demonstrated previously that an oxygen-dependent reperfusion injury occurs in the rat model of orthotopic liver transplantation [21]. Nonparenchymal cell injury characterized by loss of viability of sinusoidal endothelial cells and activation of Kupffer cells occurrs following cold storage and reperfusion which precede graft failure [5–7, 9, 18, 19]. Accordingly, a new rinse solution, Carolina Rinse, was developed to minimize reperfusion injury following cold storage [9, 12, 13]. Carolina Rinse, which is mildly acidotic [8, 14], contains extracellular inorganic ions at concentrations similar to blood; a calcium channel blocker to inhibit Kupffer cell activation [20]; and antioxidants and radical scavengers (allopurinol, glutathione and desferrioxamine) to minimize O_2 radical formation. Carolina Rinse also contains adenosine to improve the microcirculation [10] and fructose to supply energy under hypoxic conditions [1]. Indeed, by rinsing liver grafts with Carolina Rinse, postoperative enzyme release is minimized and survival is improved dramatically following orthotopic liver transplantation in models with and without rearterialization [2, 12, 13]. However, in the non-rearterialized model the efficacy of Carolina Rinse is lost when adenosine is omitted [11]. The importance of adenosine is further emphasized when donor livers are rinsed simply with Ringer's solution containing adenosine. Under these conditions, average survival time is improved as effectively as with Carolina Rinse [4]. Importantly, while adenosine is as effective as Carolina Rinse at improving survival, it does not prevent liver injury. Thus, we reasoned that a simple combination of adenosine with

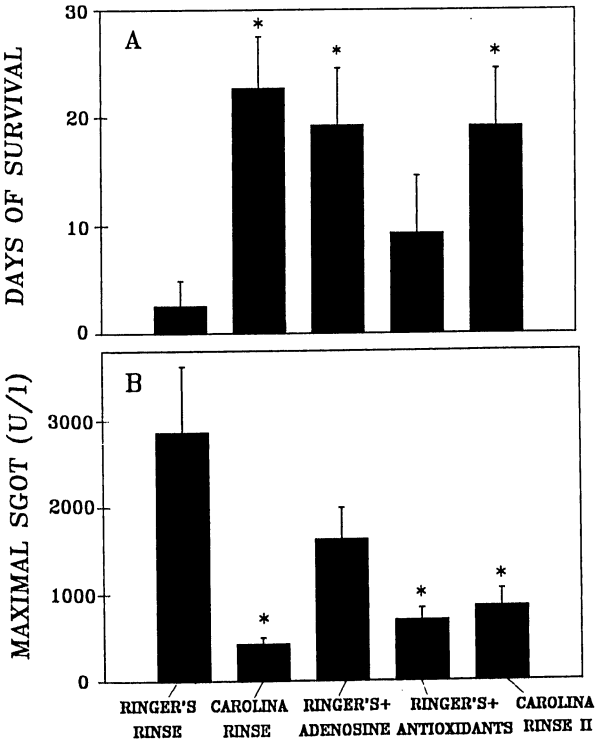

Fig. 1 A, B. Effect of Carolina Rinse II on postoperative survival. **A** Livers were rinsed and stored in UW cold storage solution at 0–4°C for 12 h (non-survival conditions). After storage, livers were rinsed with 15 ml of either cold Ringer's solution, Carolina Rinse, Ringer's with antioxidants and radical scavengers (1.0 mM allopurinol, 1.0 mM desferrioxamine, 3.0 mM glutathione), Ringer's with 0.1 mM adenosine, or Carolina Rinse II (see Table 1). Graft survival was assumed to be permanent when rats were alive 30 days postoperatively. * $P < 0.05$ for comparison with Ringer's rinse group. $n = 7$–13 per group. **B** Blood samples were collected postoperatively via the tail vein of recipient rats at 2 day intervals during the first week and at weekly intervals thereafter. SGOT activity was measured as described in Methods. Data represent maximal values observed 1–3 days postoperatively. * $P < 0.05$ compared to Ringer's rinse group. $n = 4$–6 per group

antioxidants and radical scavengers might both improve survival and minimize postoperative hepatic injury. Therefore, this study was designed to determine the effect of Carolina Rinse II, a simple solution developed to meet these criteria, on graft injury and survival following orthotopic liver transplantation in the rat.

Methods

Transplantation. Liver transplantations were performed under ether anesthesia using a technique described by Zimmermann [22] and Kamada [17]. Syngenic female Lewis rats (175–200 g) were used to eliminate immunologic interference. Briefly, livers were removed and cuffs were placed on the portal vein and subhepatic vena cava of donor livers. Grafts were stored at 0–4°C for 12 h in University of Wisconsin (UW) solution and were rinsed either with 15 ml of Ringer's solution, Ringer's solution containing adenosine (0.1 mM) or antioxidants, Carolina Rinse, or Carolina Rinse II. Subsequently, livers were implanted by connecting the suprahepatic vena cava with a running suture, inserting cuffs into appropriate vessels, and anastomosing the bile duct with an intraluminal splint. The explantation required less than 6 min, and the ischemic interval due to clamping the portal vein during the implantation procedure did not exceed 15 min. Surviving animals were sacrificed after 30 days for histology.

Serum enzymes. Following transplantation, blood samples were drawn from the tail vein at 2-day intervals during the first 7 days and at weekly intervals thereafter. Sera were separated by centrifugation and kept at −20°C for subsequent enzyme measurements. Serum glutamic oxaloacetic transaminase (SGOT) was assayed by standard enzymatic procedures [3].

Histology. Livers were fixed 24 h postoperatively with 1% paraformaldehyde in Krebs-Henseleit buffer and processed for histology. Sections were stained with hematoxalin and eosin. Liver damage was scored using a scale of 0–5 based on the degree of necrosis and 0–2 based on six structural parameters: cellular swelling, acidophilic nuclear inclusions, nuclear pyknosis, cellular deposition, cytoplasmic vacuolization, and sinusoidal dilatation (maximal score = 17). The degree of damage to the lung was expressed as a percentage of the microscopic area exhibiting infiltration of inflammatory cells.

Results

Composition of Carolina Rinse and Carolina Rinse II. Carolina Rinse II contains adenosine to improve survival and antioxidants to prevent liver injury (Table 1). The original Carolina Rinse formulation contains modified hydroxyethyl starch (pentastarch) for oncotic support against interstitial edema, antioxidants and radical scavengers, as well as extracellular inorganic ions at concentrations similar to those found in blood and Ringer's solution. Carolina Rinse also contains fructose and glucose plus insulin to diminish hypoxic injury and a dihydropyridine-type calcium channel blocker. Further, Carolina Rinse is mildly acidotic because small decreases in pH have been shown to reduce hypoxic injury to hepatocytes [8]. In contrast, Carolina Rinse II is a simple solution which only contains extracellular inorganic ions similar to Ringer's solution, adenosine, antioxidants and radical scavengers (Table 1). Like the original formulation, Carolina Rinse II is acidotic.

Effects of Carolina Rinse II on graft function and survival. After 12 h cold storage in UW solution (non-survival conditions), livers were rinsed with Ringer's solution, Carolina Rinse, Ringer's containing adenosine, Ringer's with antioxidants, or Carolina Rinse II. In the Ringer's rinse group, average survival was only 8% (Fig. 1). Similar results were obtained when grafts were rinsed with Ringer's containing antioxidants or with Carolina Rinse with adenosine deleted (data not shown). In contrast, Carolina Rinse, Ringer's containing adenosine, and Carolina Rinse II increased survival dramatically to 60–75%. Peak SGOT levels reached values around 3000 U/l in the Ringer's rinse group 24 h following implantation surgery. Surprisingly, values were also very high in the Ringer's containing adenosine group. In the Carolina Rinse group, however, the peak SGOT was only around 800 U/l. Similar results were obtained with Ringer's solution containing antioxidants and with Carolina Rinse II. Histological evaluation showed that damage to the liver and lung was also decreased 2- to 3-fold by Carolina Rinse II (Table 2). Furthermore, in livers stored under more relevant clinical conditions of survival (8 h in UW), Carolina Rinse II re-

Table 1. Composition of Carolina Rinse and Carolina Rinse II

Component	Carolina Rinse	Carolina Rinse II
NaCl	115.0 mM	102.7 mM
KCl	5.0 mM	4.0 mM
$CaCl_2$	1.3 mM	1.8 mM
KH_2PO_4	1.0 mM	–
$MgSO_4$	1.2 mM	–
Sodium Lactate	–	27.4 mM
Allopurinol	1.0 mM	1.0 mM
Desferrioxamine	1.0 mM	1.0 mM
Glutathione	3.0 mM	3.0 mM
Nicardipine	2.0 μM	–
Adenosine	1.0 mM	0.1 mM
Fructose	10.0 mM	–
Glucose	10.0 mM	–
Hydroxyethyl Starch	50.0 g/l	–
Insulin	100.0 U/l	–
MOPS	20.0 mM	–
pH	6.5	< 6.5
mosmd/l	290–305	273

Table 2. Effect of Carolina Rinse II on index of liver and lung damage. Livers were transplanted as described in Methods following 12 h (non-survival conditions) cold storage in UW solution. Rats were sacrificed 24 h postoperatively for histology. The index of liver and lung damage was scored as described in Methods. Mean ± S.E.M. for 4 livers and lungs in each group

Group	Liver damage score	Lung damage %
Ringer's Rinse	9.5 ± 0.7	72.5 ± 4.8*
Carolina Rinse II	6.9 ± 0.5*	52.0 ± 6.2*

* $P < 0.05$ for comparison with Ringer's rinse group

Table 3. Rationale for Carolina Rinse II

Addition	Hepatic mechanism (SGOT)	Extra-hepatic mechanism (Survival)
Adenosine	–	+
Antioxidants, radical scavengers	+	–
Carolina Rinse II (adenosine + antioxidants and radical scavengers)	+	+

Effective as rinse at improving (+) or not improving (–) the parameter being evaluated. All additions except Carolina Rinse II were in Ringer's solution

duced postoperative SGOT release 2- to 3-fold compared with Ringer's rinse (1255 vs 464 U/l).

Discussion

The development of UW cold storage solution extended the time of liver graft preservation for up to 30 h [16]. Primary nonfunction of livers stored in UW solution still occurs in about 15% of liver transplant patients [15]. The effects of the rinse solution on graft injury were not studied until recently and may be very important. Following cold storage, Ringer's solution is used by most transplant treams to remove potassium contained in the UW solution; however, rinsing with Ringer's solution, even for short periods of time, can cause graft edema [13]. Since an oxygen-dependent reperfusion injury occurs in the rat model of orthotopic liver transplantation [21], rinsing with well-designed solutions could reduce reperfusion injury. Accordingly, a new rinse soution, Carolina Rinse, was designed to minimize reperfusion injury following orthotopic liver transplantation. Indeed, Carolina Rinse improved graft survival from 4% to 56% and reduced maximal postoperative SGOT release 3-fold compared with Ringer's solution. Carolina Rinse also diminished postoperative sinusoidal endothelial cell damage assessed by electron microscopy [13]. Thus, Carolina Rinse is a superior alternative to Ringer's solution in vivo, protecting liver grafts from reperfusion injury.

Interestingly, removal of the calcium channel blocker or elevating the pH did not diminish the efficacy of Carolina Rinse in the non-arterialized model; however, when adenosine was omitted, Carolina Rinse no longer improved survival [10]. Further, rinsing with Ringer's containing 0.1 mM adenosine was as effective as Carolina Rinse at improving postoperative survival although postoperative SGOT values were not reduced significantly and the endothelium was ragged (Table 3). On the other hand, survival was not improved significantly by Ringer's containing antioxidants alone, yet postoperative SGOT values were decreased 4- to 5-fold. These results indicated that adenosine is an essential component of Carolina Rinse and is needed to improve survival (i.e., the extrahepatic mechanism), and that oxygen radicals may be involved in a second mechanism (i.e., the hepatic mechanism) responsible for graft injury. On reperfusion, antioxidants and radical scavengers are necessary to minimize reperfusion injury to the liver. Indeed, Carolina Rinse II, designed with these criteria in mind, improved graft survival from 8% to around 60% and reduced postoperative SGOT values nearly 3-fold compared with the control Ringer's rinse group. Since Carolina Rinse II is a simple solution composed of inexpensive ingredients, it represents a practical way to reduce postoperative reperfusion injury following live transplantation.

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