



Hypothermic Liver Machine Perfusion With EKPS-1 Solution vs Aqix RS-I Solution: In Vivo Feasibility Study in a Pig Transplantation Model

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ABSTRACT

Objective. Hypothermic machine perfusion (HMP) is superior to simple cold storage for kidney preservation. We previously observed in a porcine liver transplantation model increased tumor necrosis factor- α (TNF- α) production eventually leading to poor recipient survival after HMP using standard kidney perfusion solution (KPS-1) compared with simple cold storage. We compared two solutions for HMP preservation of the liver: enriched KPS-1 (EKPS-1) and Aqix RS-I.

Methods. Pig livers were obtained after cold flushing with histidine-tryptophan-ketoglutarate solution. Subsequently, the livers were subjected to dual-vessel perfusion with two preservation solutions: EKPS-1 ($n = 6$) and Aqix RS-I ($n = 3$). After HMP preservation and transplantation, graft and recipient survival, hepatocellular damage (aspartate aminotransferase concentration), TNF- α production, and endothelial cell damage (hyaluronic acid clearance) were recorded.

Results. No primary graft nonfunction was observed. Recipient survival at postoperative day 3 was similar in both groups (33%). Aspartate aminotransferase concentration measured in serum samples after reperfusion was similar in both groups. After reperfusion, TNF- α concentration was higher and hyaluronic acid clearance was lower in the EKPS-1 group vs the Aqix RS-I group at 60, 120, and 180 minutes (all $P < .05$).

Conclusion. Hypothermic machine perfusion provided adequate longer term graft survival. After reperfusion, TNF- α production seems to be reduced, and endothelial cell dysfunction remains pronounced with Aqix RS-1 solution compared with EKPS-1 solution.

LIVER TRANSPLANTATION is the only treatment for end-stage liver disease. Improved surgical techniques and new immunosuppressive drugs have resulted in transplantation being performed in increasing numbers of patients, with excellent results.¹ Because of increased demand, there is a shortage of donor livers. This shortage may be reduced by using expanded donor criteria.² However, simple cold storage (SCS), the standard preservation method, is insufficient for optimal preservation of organs from such donors including livers obtained from donors after cardiac death and steatotic livers.³⁻⁵

Hypothermic machine perfusion (HMP) offers an alternative method to better maintain donor organ viability. This method is efficacious in clinical kidney transplantation but needs “fine tuning” in liver transplantation. Hyperthermic machine perfusion can generate controlled perfusion of the entire organ, promoting thorough washout of blood and subsequent tissue equilibration with the preservation solu-

tion.⁶ Various solutions have been proposed for preservation of liver grafts using HMP, including Polysol,⁷ Vasosol,⁸ KPS-1 (Organ Recovery Systems, Zaventem, Belgium),⁹ and the starch-free University of Wisconsin (UW; Madison, Wisconsin) solution.¹⁰ Using a perfusion protocol similar to that for the kidney, we previously compared the outcomes of transplanted porcine livers preserved with HMP vs SCS.

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After HMP, we noted improved early hepatocyte integrity (assessed as decreased aspartate aminotransferase [AST] concentration posttransplantation) and increased production of tumor necrosis factor- α but poorer recipient survival (25% vs 83%).¹¹

In the present study, we changed the HMP protocol by adding oxygen to the perfusate and replacing KPS-1 with two other solutions: enriched KPS-1 (EKPS-1) and Aqix RS-I (Aqix Ltd, London, England). The EKPS-1 solution consists of KPS-1 plus agents expected to attenuate ischemia-reperfusion injury. Aqix RS-I, a novel non-phosphate-buffered solution for experimental organ preservation,^{12,13} contains organic buffers, amino acids, and metabolic substrates.

MATERIALS AND METHODS

Porcine livers procured as described previously¹⁴ were preserved for 4 hours with HMP. Livers were perfused through the portal vein (3–5 mm Hg; 0.25 mL/g of liver per minute) and through the hepatic artery (20 mm Hg). The perfusion settings enabled limitation of maximal flow on the portal vein side and of maximal pressure on the hepatic artery side. The machine perfusion solution was oxygenated (PO₂, 310 \pm 67 mm Hg) using a pediatric non-coated oxygenator (Minimax; Medtronic, Inc, Minneapolis, Minnesota). Two groups were compared, one with EKPS-1 (n = 6) and one with Aqix RS-I (n = 3), using a specifically designed liver HMP device (Organ Recovery Systems, Zaventem, Belgium). The composition of KPS-1 and agents added to produce EKPS-1, and of Aqix RS-I are given in Table 1. Temperature was maintained between 5°C and 8°C. The machine perfusion solution (volume, 2 L) was recirculating. Subsequently, the livers were transplanted into recipient animals. At postoperative day (POD) 3, graft function and survival were recorded, and surviving animals were sacrificed under general anesthesia.

Using a central catheter placed in the internal jugular vein, venous blood samples were obtained before laparotomy (baseline) in donors and recipients, after HMP preservation, at the end of the

anhepatic phase (at 0 minutes), after reperfusion (at 15, 60, 120, and 180 minutes), and daily thereafter until POD 3. After centrifugation of whole blood (10 minutes at 3000g), plasma or serum samples were frozen and stored at –25°C until analysis.

Standard absorption techniques were used to determine the release of aspartate aminotransferase (AST) in serum. TNF- α was measured in plasma using a porcine-specific enzyme-linked immunosorbent assay (R & D Systems, Abingdon, England). All standard and control samples were assayed in duplicate. Hyaluronic acid, a protein cleared solely by liver sinusoidal endothelial cells, was measured in plasma using a protein-binding assay (Corgenix UK Ltd, Peterborough, England). Reference solutions prepared from rooster comb hyaluronic acid were used to estimate concentrations (in nanograms per milliliter). All standard and control samples were assayed in duplicate.

Statistical Analysis

Analysis of variance and the Tukey post hoc test were performed. Data are expressed as mean (SD). $P \leq .05$ was considered statistically significant.

RESULTS

No primary graft nonfunction was observed. Recipient survival at POD 3 was similar in both groups (33%) (Fig 1A).

AST, a marker of hepatocellular integrity, was measured in serum samples after reperfusion and was found to be similar in both groups (Fig 1B). At POD 1, AST peaked in the surviving animals in both groups (POD 1 to POD 3: EKPS-1, n = 2; Aqix RS-I, n = 1).

TNF- α , a marker of inflammation, was higher in the EKPS-1 group compared with the Aqix RS-I group at 60 minutes (2233 [1294] vs 120 [114] pg/mL), 120 minutes (2161 [981] vs 240 [196] pg/mL), and 180 minutes (948 [484] vs 162 [122] pg/mL) (all $P < .05$) (Fig 1C). After POD 1, TNF- α

Table 1. Composition of KPS-1, EKPS-1, and Aqix RS-I

KPS-1	EKPS-1	Aqix RS-I
Sodium, 100 mmol/L	KPS-1	Sodium, 110 mmol/L
Potassium, 25 mmol/L	Deferoxamine mesylate salt, 1 mmol/L	Potassium, 5 mmol/L
Magnesium, 5 mmol/L	Insulin, 100 U/L	Calcium, 1.25 mmol/L
Calcium, 0.5 mmol/L	Misoprostol, 70 μ g/kg	Magnesium, 0.45 mmol/L
Chloride, 1 mmol/L	Trolox,* 5 \times 10 ⁵ mmol/L	Sodium bicarbonate, 25 mmol/L
Phosphate, 25 mmol/L	Superoxide dismutase, 32,000 U/L	BES, 5 mmol/L
HEPES, 10 mmol/L	Catalase, 137,500 U/L	D-Glucose
Glutathione, 3 mmol/L	Pentoxifylline, 1 g/L	Calcium chloride, 1.25 mmol/L
Gluconate, 85 mmol/L	L-Glutamic acid, 3 mmol/L	Glycerol, 0.11 mmol/L
Mannitol, 30 mmol/L	L-Alanine, 1 mmol/L	L-Glutamate, 0.30 mmol/L
D-Glucose, 10 mmol/L	L-Glycine, 3 mmol/L	L-Glutamine, 0.40 mmol/L
Ribose, 5 mmol/L	L-Arginine, 3 mmol/L	L-Aspartate, 0.02 mmol/L
Adenine, 5 mmol/L	Reduced glutathione, 3 mmol/L	L-Carnitine, 0.05 mmol/L
HES, 50 g/L	Dibutyl cAMP, 2 μ mol/L	Choline chloride, 0.05 mmol/L
	Glucose, 21 mmol/L	TTP (cocarboxylase), 40 nmol/L
	Adenosine, 2 mmol/L	Insulin, 28 mU
	Fructose, 10 mmol/L	
	S-adenosylmethionine, 100 μ mol/L	

Abbreviations: BES, balanced electrolyte solution; cAMP, cyclic adenosine monophosphate; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic [acid]; HES, hydroxyethyl starch; TTP, thiamine triphosphate.

*Trolox (Hoffman-LaRoche, Nutley, New Jersey) is the tradename for 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

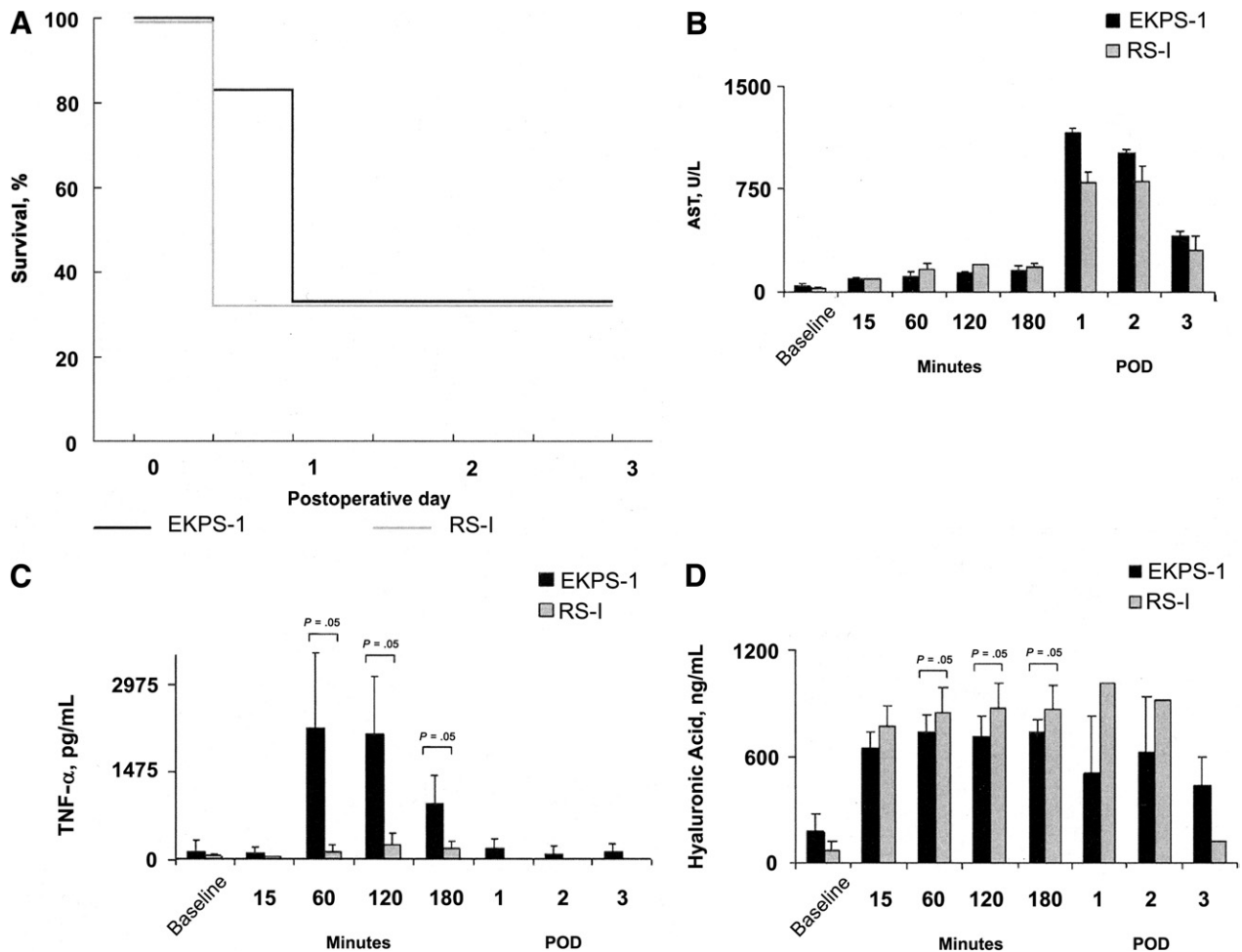


Fig 1. Liver transplantation with hypothermic liver machine perfusion with preservation with perfusion solutions EKPS-1 and Aqix RS-I. A, Recipient survival at postoperative day 3 was similar in both groups (33%). B, Aspartate aminotransferase (AST) concentration measured in serum samples after reperfusion was similar in both groups. From postoperative day (POD) 1 onward, AST concentration increased in both groups compared with previous time points. C, Tumor necrosis factor- α (TNF- α) after reperfusion was higher in the EKPS-1 group compared with the Aqix RS-I group at 60, 120, and 180 minutes. After POD 1, TNF- α concentration decreased. D, Hyaluronic acid concentration was lower after reperfusion in the EKPS-1 group compared with the Aqix RS-I group at 60, 120, and 180 minutes.

concentration decreased (POD 1 to POD 3: EKPS-1, $n = 2$; Aqix RS-I, $n = 1$) compared with previous time points.

Hyaluronic acid, a marker of sinusoidal endothelial cell function, was lower after reperfusion in the EKPS-1 group compared with the Aqix RS-I group at 60 minutes (738 [97] vs 770 [117] ng/mL), 120 minutes (712 [116] vs 845 [143] ng/mL), and 180 minutes (736 [77] vs 876 [134] ng/mL) (all $P < .05$) (Fig 1D).

DISCUSSION

Hypothermic machine perfusion is superior to SCS for kidney preservation¹⁵ and, as a consequence, may help increase future kidney supply. We hypothesized that livers would be better preserved using HMP compared with SCS. Many studies in rodents have suggested that HMP of the liver is superior to SCS; however, data from in vivo large

animal transplantation models are rare.^{16,17} In addition, there is controversy and ongoing research as to the ideal HMP parameters and settings including flow, oxygen, pulsatility, machine perfusion solution, and optimal temperature.¹⁸

In a previous in vivo study using parameters derived from standard kidney HMP (nonoxygenated perfusion with KPS-1), porcine livers transplanted after 4 hours of HMP compared with SCS demonstrated increased TNF- α production, poorer hyaluronic acid clearance, and eventually, reduced survival.¹¹ In an ex vivo porcine model, we documented that liver preservation using HMP could be improved by adding oxygen to the perfusate and by lowering the perfusion pressure. Under these conditions, the adenosine triphosphate concentration remained stable and the livers were morphologically well preserved.⁹ We concluded, in accord with others, that liver HMP, unlike kidney HMP, requires an oxygenated

perfusion solution.¹⁰ The positive effect of lowering the perfusion pressure is possibly related to reduced shear stress on endothelial cells, as reported by others.¹⁹

We then applied low perfusion pressure and oxygenation in an *in vivo* transplantation model. In addition, we tested two new preservation solutions, EKPS-1 and Aqix RS-I. The EKPS-1 was formulated by the addition to standard KPS-1 of reactive oxygen species inhibitors, a free iron chelator, nutrients, and other agents (Table 1), to support metabolism, improve microcirculation, and stabilize Kupffer cells. Aqix RS-I was also tested on the basis of its more physiologic characteristics (organic buffers, amino acids, and metabolic substrates) and its promising results in experimental heart¹² and kidney¹³ machine perfusion. We analyzed graft and recipient survival and certain surrogates of parenchymal integrity (AST), inflammation (TNF- α), and endothelial cell function (hyaluronic acid clearance).

Parenchymal hepatocellular integrity was similar with both solutions. It has been reported that HMP offers better hepatocellular preservation compared with SCS.^{20,21} We speculate that this benefit is not necessarily influenced by the type of perfusion solution but is merely the result of the “mechanical” effect of HMP, which enables better penetration and, therefore, better preservation of the microcirculation and the parenchyma. The morphologic characteristics, as assessed by architectural integrity, congestion, neutrophil influx, coagulation necrosis, sinusoidal dilatation, and parenchymal vacuoles, were similar with both preservation solutions (data not shown). The production of TNF- α , a proinflammatory cytokine primarily secreted by Kupffer cells, was surprisingly higher in EKPS-1 than in Aqix RS-I. This observation was unexpected because EKPS included a series of additives (pentoxifylline and glycine) designed to improve Kupffer cell preservation. We hypothesize that the TNF- α production was probably related to the KPS-1, although the mechanism requires clarification. High systemic levels of TNF- α have serious implications. In addition to properties in the liver that induce apoptosis, adverse effects have been reported in lung²² in a systemic inflammatory response syndrome. The high TNF- α release may have contributed to recipient deaths in the EKPS-1 group. Early endothelial cell dysfunction, as measured by hyaluronic acid clearance, was present in both groups but was more pronounced in the Aqix RS-I group. Endothelial cells are not well preserved by HMP when the pressure of the solution is too high²⁰; however, in our experiments, the pressure was low. Reasons for more pronounced endothelial cell dysfunction in livers preserved with Aqix RS-I need clarification, and caution must be used in interpreting these data because of the low number of animals in the Aqix RS-I group.

Compared with our previous *in vivo* HMP results using nonoxygenated KPS-1, we did not observe a major improvement in survival after transplantation of HMP-preserved livers even after lowering the perfusion pressure, adding oxygen to the solution, and modifying the perfusion solution. This is in contrast to our observations in *ex vivo*

models that oxygenation and pressure lowering are beneficial in optimizing preservation. This contradiction indicates the necessity of using *in vivo* transplantation models in HMP research before reaching definitive conclusions or applying this technology clinically. The finding that TNF- α release was abrogated in livers preserved with Aqix RS-1 is important because the phenomenon of Kupffer cell activation has been a hurdle in successful application of HMP in our laboratory.

In conclusion, in an *in vivo* transplantation model, HMP of porcine livers using two perfusion solutions (other than standard KPS-1 used for kidney perfusion) is feasible and primary graft nonfunction is not encountered. However, in the setting described herein, HMP is not capable of reproducibly providing adequate long-term survival. Ongoing research using *in vivo* transplantation models is necessary to determine the best perfusion settings, including optimal temperature, and eventually, to unmask the benefits of machine perfusion of liver grafts.

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