Ex vivo evaluation of porcine livers post-hypothermic machine perfusion at 4-6°C and 12-14°C

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Abstract

Background: The goal of our research is the development of a clinical hypothermic machine perfusion method and device for liver preservation during storage and transport prior to transplantation. The purpose of this study was comparison of two hypothermic temperature ranges.

Methods: Heart beating donor pig livers with 2h of static cold storage on ice were employed. Thirteen experimental livers were perfused, at either 4-6°C or 12-14°C with oxygenation, employing a prototype device. They were compared with six control 2h static cold stored livers during normothermic ex vivo blood perfusion. Physiological measurements and biochemical perfusate analyses were performed to assess the impact of storage conditions on liver functions. Statistics were assessed by one way analysis of variance, $p < 0.05$ was regarded as significant.

Results: During hypothermic perfusion perfusate solutes (Na^+ , K^+ and Ca^{++}) varied little with time. A single 12-14°C perfused liver exhibited a Ca^{++} spike at 22 hours. Temperature dependent metabolic activity was observed in both groups. Lactate levels were $\leq 4.5 \text{ mmol/L}$ for 22h. During normothermic ex vivo testing most controls and perfusion-treated experimental livers produced bile within an hour. 10h perfusion liver lactate dehydrogenase, hyaluronic acid uptake, and total bile production were not significantly different from controls. There were trends for albumin, glucose and lactate. Significantly different Factor V, indocyanine green clearance, and blood urinary nitrogen concentrations were observed in both 10h perfusion groups. Bilirubin kinetics was perturbed in all perfusion groups, however the peak concentrations in the 10h perfusion groups were not significantly different, while the 22h 12-14°C perfusion group was significantly less than controls. A significant difference in alanine aminotransferase values between the 2 perfusion groups, was observed, however this may be due to washout during 12-14°C perfusion. Lactate dehydrogenase was doubled in both perfusion groups at 22h and individual 22h livers exhibited other assay values outside control and 10h perfusion ranges.

Conclusions: Significant differences were observed between controls and both perfused groups during post hypothermic storage *ex vivo* assessment. These results suggest that both temperature ranges tested, with further optimization, may be suitable for liver support for up to 10h of oxygenated hypothermic perfusion. Since there were no clear advantages to 12-14°C perfusion we will continue development of a 4-6°C perfusion device for storage and transport of livers for transplantation.

Introduction

There are currently two approaches for preservation of most transplantable organs, either static or perfused storage. Simple static cold storage (SCS) is the main method for organ storage, while hypothermic machine perfusion (HMP) and other perfusion-based methods such as normothermic machine perfusion and oxygen persufflation comprise the methods for perfused preservation. SCS and HMP are approved clinically for kidneys and only SCS for other organs. The remaining methods are in various stages of pre-clinical and early clinical studies. Profound kidney HMP at $< 15^\circ\text{C}$ has already demonstrated significant clinical improvements in the quality of kidney preservation and patient treatment [1,2,3] including the value of perfusate biomarkers [3]. In contrast, current clinical practice for liver preservation is to flush the liver with a preservation solution and then store it at $0-4^\circ\text{C}$.

Liver HMP has lagged behind kidney. In preliminary studies of porcine liver HMP at $4-6^\circ\text{C}$ without oxygenation we previously

observed benefits of perfusion compared with SCS that were attributed to better sinusoidal endothelial cell function and homogeneous sinusoidal perfusion [4]. Monbaliu *et al.* [5] performed a porcine transplant study using the same prototype machine at $4-6^\circ\text{C}$ without oxygenation. Four hours of SCS ($n=6$) was compared with 4 hours of hypothermic machine perfusion ($n=8$) by evaluating post transplant recipient graft survival, liver damage and hepatocellular functions. Three-day survival was 83% (5/6) in the cold storage group and only 25% (2/8) in the hypothermic machine perfusion group. It is possible that oxygen deprivation during profound HMP may have played a major role in these generally poor results. In the early 1960s, as liver transplantation was approaching clinical reality, Sicular and Moore [6] investigated various methods of cooling and preserving organs by measuring glucose metabolism and carbon dioxide production. They reported good maintenance of function in a liver perfused with an acellular, oxygenated perfusate at 15°C , just on the edge of the profound hypothermia temperature range. Several studies demonstrated that perfusion with oxygenated solutions prevented ATP loss and avoided the injurious ischemic cascades that are set into motion after ATP depletion [7,8,9,10]. The role of oxygenation has also been supported by other studies [11,12,13,14]. More recently,

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Guarrera *et al.* [15] compared SCS (n=3) with hypothermic machine perfusion (n=3), by perfusing both the portal vein and hepatic artery in porcine livers. Post transplant liver function results were similar between groups, with all animals surviving for 5 days. Guarrera *et al.* [16] also reported the first successful clinical HMP liver study. The experimental profound HMP liver literature provides a rather mixed review but the main take home message is that oxygenation may be beneficial [17]. However, the most successful study to date [16] did not use oxygen.

Support for the concept of liver preservation above 8°C can be drawn from the work of Kruuv *et al.* [18,19] using tissue culture cells as an experimental model. One popular hypothesis of hypothermic or chilling injury states that at a certain critical temperature the membrane lipids undergo a transition from a liquid-crystalline to a solid gel state. The two main consequences of the transition are thought to eventually result in cell injury due to an increase in membrane permeability and an increase in the activation energy of membrane bound enzymes. Kruuv *et al.* showed that the Arrhenius plot of inactivation (killing) rates of cells exposed to reduced temperatures changes slope at approximately 7-8°C implying that there are distinct mechanisms of hypothermic inactivation above and below this transition temperature. In the range of 8 to 25°C, the activation energy from the Arrhenius plot for control cells

is about 15 kcal/mol which falls within the range of temperature coefficients of metabolic processes (10-30 kcal/mol) and much lower than that for protein denaturation. Below 8°C, the magnitude of the apparent activation energy is large (-61 kcal/mol). These values have been interpreted to suggest that unbalanced metabolism is probably the rate limiting step for hypothermic inactivation in the higher temperature range (above 8°C), and membrane lipid phase transition or cold denaturation of a critical protein is likely to be responsible for the strong temperature dependence in the lower range (below 7-8°C). Thus it is apparent that the optimum temperature for hypothermic storage may depend upon a variety of factors involving the interaction of hypothermia, the nature of the cell or organ, and the chemical composition of its environment. Further evidence supporting the use of preservation temperatures >10°C was that maximal myocardial preservation during ischemic arrest is best achieved in the range of 10 to 20°C. For example, metabolic recovery was best when the myocardium was kept at 10 to 15°C with rapid reperfusion recovery of high energy phosphates and glycogen, compared with metabolic deterioration at 4°C [20].

Therefore, we investigated oxygenated HMP in two hypothermic temperature ranges, 4-6°C and 12-14°C, employing a prototype device (Figure. 1). Support for the concept of liver preservation above 8°C was drawn from the work of Kruuv *et al.* [18,19] who

Figure. 1: Prototype liver device during HMP. The pediatric oxygenator & heat exchanger are out of view on the left side. This design is based upon two Lifeport® Kidney transporters pump decks (left and right back) combined with independent controls and a single large organ cassette and bath (front, center).

demonstrated that there may be different mechanisms of hypothermic inactivation of cells above and below 7-8°C. Cells preserved below this temperature range having a shorter shelf-life. Support for utilization of preservation temperatures above 7-8°C, between 10 and 15°C, can be found in older literature [6,20]. We used 12-14°C as our higher target preservation temperature range in order to have a significant temperature buffer to minimize temperature deviations above 15°C, where oxygen supply may become limiting in the absence of an oxygen carrier.

Materials & Methods

Nineteen adult domestic Yorkshire cross farm pigs (25-30 kg) were used as heart beating donors. All aspects of this research involving animals was approved by the Medical University of South Carolina Animal Care and Use Committee prior to initiation. The animal care and handling complied with the "Principles of Laboratory Animal Care" as formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" published by the National Research Council [21]. The animals were weighed and pre-anesthetized with a mixture of ketamine (22 mg/kg), acepromazine (1.1 mg/kg) and atropine (0.05 mg/kg) given IM. After establishing an ECG, the pigs were intubated, and placed on isoflurane anesthesia at 1.5-2%. The pigs were anticoagulated with heparin (400 IU/kg, IV). After a midline incision from the xiphoid process to the pubis, the dorsal aorta was cannulated below the liver, and clamped off above the liver. The livers were perfused with 4L of Lactated Ringers, flushed with Belzer's machine perfusion solution, excised and placed in a plastic bag on ice for 2h during transport and preparation for studies. Following liver removal, the donor's heparinized blood was collected for use in the perfusate during post-HMP normothermic evaluation. The pig was euthanized by exsanguination under anesthesia.

Thirteen experimental HMP livers were compared with six 2h static cold stored control livers using a battery of cell/tissue damage and function assays by periodic sampling during 3h normothermic *ex vivo* perfusions (Figure. 2). The liver perfusion device was a prototype hypothermic liver perfusion machine, based upon the Lifeport Kidney transporter (Organ Recovery Systems, Inc., Itasca, IL) primed with the perfusion solution, Belzer's machine perfusion solution (KPS-1, Organ Recovery Systems, Inc., Itasca, IL), at a steady-state hypothermic temperature, either 4-6°C or 12-14°C. Organ cassettes with the afferent flow circuit tubing and disposable sterile filters were used (20 µm, Meissner) to maintain the sterility of the perfusate and retain residual red blood cells. The livers were continuously perfused, for 10 or 22h at the desired temperature at 8mmHg venous and 25mmHg arterial pressures. The organ bath temperature, perfusion pressure and flow rate were monitored and recorded. Medical grade 95% O₂ with 5% CO₂ was equilibrated with the perfusion solution to elevate its pO₂ to ~500 mmHg prior to entering the liver. Liver weights were documented before and after SCS, HMP and normothermic perfusion.

At the conclusion of HMP or SCS period the livers were evaluated

on an *ex vivo* normothermic liver test circuit. The perfusion circuit included the liver perfusion device with an attached pediatric oxygenator and heat exchanger. The heat exchanger was set to maintain the perfusate in the bath at ~37°C. The perfusate for the isolated perfusion system was 2.5L of Krebs-Henseleit Buffer with 20-25% washed red blood cells from the donor pig combined with expired banked human blood to achieve the desired hematocrit. The circuit was primed prior to reperfusion and pH and pCO₂ were adjusted to normal physiological range. The liver was then placed in the perfusion circuit and tested over a period of 3h. The pressure in the portal vein (PV) was set to 18mmHg and the hepatic artery (HA) to 60-70 mmHg.

Perfusate samples were collected frequently during organ preservation and every 30min during reperfusion. A reference baseline sample was collected from the organ bath prior to HMP and normothermic assessment. Samples for pCO₂, pO₂, electrolytes (Na⁺, K⁺, Ca⁺⁺, NH₄⁺⁺) and metabolites (lactate and glucose) were tested immediately on a Bioprofile400. Samples collected during normothermic assessment were measured for lactate dehydrogenase and alanine aminotransferase using colorimetric diagnostic kits (Abcam, Bioassay Systems). Specific liver functions were evaluated during the reperfusion period including bile production, Factor V, indocyanine green clearance, and total bile bilirubin. Factor V production was measured by enzyme-linked immunosorbent assay (ELISA) (Enzyme Research labs) [22]. Bile production and indocyanine secretion [23] are commonly used in our laboratories as indicators of liver functions following stress and preservation. Bile was collected every 30 minutes. Total bile bilirubin was measured using a diagnostic kit (Pointe Scientific) [24,25]. Indocyanine green was premixed in the perfusate at a final concentration of 10mg/L. Bile collected during reperfusion was tested for the presence of indocyanine green (780nm) with a UV-visible light spectrophotometer (Spectramax Gemini EM, Molecular Devices) [26,27]. Hyaluronic acid (HYA) uptake [28,29] was used to assess endothelial cell damage. 150ug/L of HYA was included in the perfusate. The concentration of HYA in the perfusate samples was determined by ELISA (R&D Systems).

Statistical Methods

The 10 hour experiments were repeated four times and the 22 hour experiments 2-3 times. Statistical differences were assessed by one way analysis of variance (ANOVA). P-values <0.05 were regarded as significant.

Results

Porcine livers were compared above and below 8°C. After HMP mean liver weights stayed relatively constant (±2.2%) for 10h of HMP with not significantly different means of 5 and 16% weight gain at 4-6°C and 12-14°C for 22h of HMP, respectively. Outflow solutes (Na⁺, K⁺ and Ca⁺⁺) varied little with perfusion time. A single 12-14°C HMP liver exhibited a 3-fold 0.93 mmoles Ca⁺⁺/L spike at 22 hours. Mean lactate levels were ≤4.5mmol/L. Active oxygenation in both 4-6 and 12-14°C HMP groups produced mean pO₂ ranges of 271-359 and 60-319 mmHg in the perfusate entering the liver, respectively,

during long term HMP. The pCO_2 levels gradually increased to mean values of 13.9 and 25.2 mmHg ($p<0.05$), respectively. The analyses performed during hypothermic perfusion are summarized in Table 1.

At the conclusion of the experimental HMP phase, the livers were tested on a normothermic circuit. The background results are shown in Table 2 and the biochemical results in Table 3. HMP-treated livers functioned immediately during *ex vivo* testing and demonstrated excellent bile production. There were no significant differences in liver weights after *ex vivo* testing, the mean weights were increased by 18-35% compared to 29% in the SCS control livers. After 10h of HMP there were no statistically significant differences in livers with regard to lactate dehydrogenase, hyaluronic acid uptake, and total bile production on the normothermic test circuit relative to controls or between HMP groups. There were trends in all HMP groups for albumin, glucose concentrations and lactate that did not achieve statistical significance. Lactate dehydrogenase was doubled in both perfusion groups at 22h and individual 22h livers exhibited other assay values outside control and 10h perfusion ranges (Table 3).

Significantly less Factor V production (Figure 3A), greater indocyanine green clearance (Figure 3B), and less blood urea nitrogen concentrations (Figure 3D) were observed in both 10h HMP groups ($p<0.05$) compared with the controls. The decreased Factor V was the only function that was significantly lower than fresh controls in both HMP groups. ALT was significantly increased in the 4-6°C group and decreased in the 12-14°C group, however for reasons discussed later we discount the last observation. The ICG clearance was better in the HMP groups than in controls. Bilirubin kinetics were perturbed in all HMP groups compared with controls (Figure 3C), however the peak concentrations achieved in the 10h HMP groups were not significantly different, while the 22h 12-14°C HMP group was significantly less than the control group ($p<0.05$).

Table 1. Overview of Hypothermic Machine Perfusion Data (ranges)

Parameter	4-6°C	12-14°C
Physical Measurements		
Flow Rate, Hepatic Artery (ml/min)	10.7-42	16.4-35
Flow Rate, Portal Vein (ml/min)	102-137	108-185
Resistance, Hepatic Artery (mmHg ÷ ml/min)	1.42-0.59	1.4-0.77
Resistance, Portal Vein (mmHg ÷ ml/min)	±0.05 steady	decreasing from 0.41-0.30
Blood Gases and General Chemistry		
pH (units)	7.21-7.08	7.2-7.0
pCO_2 (in, mmHg)	5.8-14.7	5.9-24.4
pCO_2 (out, mmHg)	8.53-13.9	9.45-25.2
pO_2 (in, mmHg)	216-538	324-533
pO_2 (out, mmHg)	271-359	60-319
Na^+ (mmoles/L)	89.6-96.3	87.1-85.2
K^+ (mmoles/L)	25.6-27.6-23.2	28.8-32.2-25.6
Ca^{++} (mmoles/L)	0.19-0.24	0.2- 0.93
Glucose (out, mg/dL)	275-333	274-441
Lactate (mmoles/L)	2.8- 1.0	2.8-4.2

Static Storage

Heart-beating donor	<i>In situ</i> topical cooling & Retrograde flush	Transport Back to lab	Evaluation
Baseline	4°C < 30 minutes	On ice 2 hours	37°C 3 hours

Perfusion Preservation

Heart-beating donor	<i>In situ</i> topical cooling & Retrograde flush	Transport Back to lab	Hypothermic machine perfusion preservation	Evaluation
Baseline	4°C < 30 minutes	On ice 2 hours	4-6°C or 12-14°C 10 or 24 hours	37°C 3 hours

Figure 2: Experimental evaluation and measurement scheme

Table 2. Overview of *Ex Vivo* Normothermic Perfusion Data (ranges during testing at 37°C)

Parameter	Control	10hours		22 hours	
	(2h static)	4-6°C	12-14°C	4-6°C	12-14°C
Physical Measurements					
Flow Rate, Hepatic Artery (ml/min)	57-89	99-119	78-103	104-108	129-149
Flow Rate, Portal Vein (ml/min)	291--351	265-379	418-511	242-228	214-286
Resistance, Hepatic Artery (mmHg ÷ ml/min)	1.08-0.69	0.52-0.56	0.97-0.57	0.55-0.56	0.53-0.40
Resistance, Portal Vein (mmHg ÷ ml/min)	0.065-0.048	0.09-0.05	0.058-33	0.07-0.07	0.09-0.047
Blood Gases and General Chemistry					
pH (units)	7.5-7.0	7.3-7.0	7.3-7.0	7.3-7.0	7.2-7.0
pCO ₂ (in, mmHg) PV	13.3-107	15-100	16-100	10-92	26-89
pCO ₂ (out, mmHg)	17-120	21-109	22-107	14-107	30-98
pO ₂ (in, mmHg) PV	357-512	358-419	407-556	43-419	249-406
pO ₂ (out, mmHg)	41-76	38-81	30-171	22-60	41-63
Na ⁺ (mmoles/L)	145-149	146-160	147-153	144-162	145-157
K ⁺ (mmoles/L)	7-6.9	3.8-1.9	4.7-3.3	3.5-1.84	6.7-7.0
Ca ⁺⁺ (mmoles/L)	0.81-0.86	0.73-8.0	0.66-0.79	0.66-0.76	0.72-0.73

Discussion

Analyses made of the perfusate during HMP revealed no significant differences between the two profound HMP groups during 10h of HMP, at the longer time point, 22h, an individual 12-14°C demonstrated a CA⁺⁺ spike (Table 1). Normothermic *ex vivo* evaluation revealed that there were no statistically significant differences between HMP groups for liver lactate dehydrogenase, hyaluronic acid uptake, total bile production albumin, glucose, lactate, Factor V production, indocyanine green clearance, BUN and ICG clearance. *Ex vivo* evaluation after 22h of HMP generally produced more variable results. Lactate dehydrogenase concentrations were doubled in both HMP groups, and the 22h 12-14°C HMP bilirubin release was significantly less than the control group (p<0.05). Individual livers exhibited other assay values well outside control and 10h HMP ranges leading to the conclusion that our HMP perfusion conditions had not been optimized for longer periods. Future studies are planned in which Vasosol, a formulation consisting of antioxidants, vasodilators and metabolic substrates, will be added to the perfusate to minimize reperfusion injury based upon the excellent outcomes of human liver HMP reported by Guarrera [16]. Antioxidants may be required since oxygenated livers are potentially at risk for oxidant damage.

During *ex vivo* testing by normothermic oxygenated blood perfusion all groups became edematous and trapped erythrocytes suggesting that adding inhibitors of vasoconstriction and reperfusion injury during HMP and or reperfusion may have significant benefits. Comparisons were made with heart beating liver controls without warm ischemia and 2h of cold ischemia (Figure 2), these organs are within procurement parameters that result in successful porcine liver transplantation [5,15]. Retention of endothelial function was

Table 3. *Ex Vivo* Function and Viability Assays after 3h of Normothermic Perfusion

Assay	Control	10hours		22 hours	
		4-6°C	12-14°C	4-6°C	12-14°C
ALT (IU/mL)	40±7	71±5 [‡]	15±2 [‡]	68±14	23±10
Factor V (µg/mL)	39±2	31±3 [‡]	30±2 [‡]	28±5 [*]	29±5 [*]
Albumin (µg/mL)	147±24	115±48	75±27 [*]	64±35	88±45
Bilirubin(peak,mg/dL))	0.91±0.29	0.28±0.2 [*]	0.84±0.67 [*]	0.36±0.18 [*]	None [‡]
Hyaluronic acid	>99% cleared	>99% cleared	>99% cleared	>99% cleared	>99% cleared
LDH (ng/mL)	272±6	336±74	269±9	635±85 ^{**}	602±15 ^{**}
ICG (µg/mL of bile)	902±23	1025±4 [‡]	1060±70 [‡]	836±29	802±3
BUN (mg/dL)	7±0.5	4.5±0.7 [‡]	3.7±0.3 [‡]	4±0	4.5±0.4
Lactate (mmole/L)	3.7±1	7.8±1.8	6.6±1	9.2	4.5±4
Glucose (mg/dL)	275±62	534±145	593±145	409	203±47
Bile (mL, total)	7±2.1	8.1±1.1	10.2±2.4	5.4	8.9±4.9

*Production kinetics did not match the controls (see Fig. 3), **Pink[‡]** significantly different control versus perfused group (p<0.05), **Pink^{*}** significantly different comparing perfused groups (p<0.05), Black indicates trends. **combined 22h HMP groups statistically different compared with other groups (p<0.05).

Figure 3: Time course examples from perfusate and bile analyses - **A)** Factor V; **B)** ICG; **C)** Bilirubin; and **D)** BUN test results

demonstrated by effective hyaluronic acid clearance in both HMP groups compared with controls (Table 3). There was a significantly lower BUN value in both HMP groups. This was probably due to loss of BUN from the livers during the hypothermic perfusion phase. There was only one assay, ALT, that demonstrated significant differences comparing the two temperatures and the 12-14°C HMP group was also significantly lower than the fresh controls. However, because the ALT values were lower than fresh controls we believe that ALT washout from the liver may have occurred during the 12-14°C perfusion period. In subsequent research where livers were perfused at 18-22°C we have observed release of ALT (Brockbank *et al.*, ongoing unpublished studies). Therefore, we have discounted this difference in the present study. We plan to evaluate the hypothermic perfusion solutions for ALT release in future studies to permit re-evaluation of our decision to discount the 12-14°C perfusion ALT values. It should be noted that this is a work in progress, this is the reason for the large number of surrogate end points for organ function assessed. Eventually, it is anticipated that a subset of end points will be justified by correlation with *in vivo* performance reducing the number of assays performed during *ex vivo* testing. In the mean time the use of heart beating donor controls with very short term static cold ischemia (2h) before normothermic assessment is a relevant comparison because these control livers should function well *in vivo* post-transplantation [5,15]. Overall, the 4-6°C HMP treatment group appeared more consistent

although not statistically different. Furthermore, it is likely that brief temperature excursions, during 4-6°C HMP, as high as 12-14°C will have minimal if any impact upon organ function.

Several solutions have been employed for hypothermic perfusion of livers including variations on University of Wisconsin (UW) solution, modified HTK solution, oxygenated Krebs-Henseleit solution, and Celisior- Hydroxyethyl starch. Three new solutions that are being tested are Vasosol [16], Polysol (Doorzand Medical Innovations, Amsterdam, The Netherlands) and Lifer an acellular oxygen carrying solution being developed by Life Blood Medical, Inc., which they claim has provided good *in vitro* function data employing a working heart model for 10 hour slow perfusion preservation of guinea pig hearts at room temperature without added oxygen [30]. More recently, a rat liver study with oxygenation was reported comparing several temperatures with the best results at room temperature [31]. We employed KPS-1 (Kidney Perfusion Solution One), a variant of University of Wisconsin solution commonly known as Belzer's Machine Perfusion Solution, oxygenated and supplemented as described in the materials and methods section. Even though KPS-1 does not contain an active oxygen carrier, it carried adequate oxygen for both profound hypothermic temperatures, 4-6°C and 12-14°C, investigated in the present study. It is anticipated that we would require an oxygen carrier at higher temperatures where metabolism is closer to that observed under physiologic conditions.

We are aware of three other liver perfusion devices being developed commercially [32,33,34]. Two systems are traditional hypothermic 4-6°C perfusion devices, the Groningen hypothermic liver perfusion pump [32] and an oxygenated device named the Airdrive (Doorzand Medical Innovations, Amsterdam, The Netherlands) [33]. The third is the POPS device of TransMedics, Inc. In contrast to the others, the POPS device is designed to operate at 37°C and it is being studied at the University of Chicago and Kings College, London. No significant in vitro or transplantation results, either animal or human, have been presented to date. The device is basically a cardio-pulmonary bypass device on a cart that weighs at least 50 lbs and has only 2-4 hours of independent battery life. The liver circuit uses a raised expansion chamber to provide non-pulsatile flow to the portal vein. The device is clearly not portable between transplant centers. Aside from the potential advantages of normothermic perfusion preservation over cold storage [35,36,37], this demanding technique will evidently lead to high costs and, due to its complexity, will place a significant burden on the workload of transplant personnel. For these reasons it is uncertain whether this technique will really take off in clinical organ preservation [34]. Hypothermia during perfusion requires less oxygenation, lower flow rates and less power, which can be supplied by 24h rechargeable batteries. An oxygen carrier is not necessary to achieve adequate oxygen delivery during profound hypothermic preservation [10]. Conversely, normothermic perfusion without adequate oxygenation creates inferior results to simple cold storage [38]. Perfusion at 37°C may provide an opportunity for the transplant team to evaluate the liver, prior to bringing the patient to the operating room, and restore normothermic metabolism prior to implantation. Profound hypothermic perfusion has better potential for simple and reliable clinical application because not only are the metabolic demands of the organ much lower, but safety concerns are also reduced if sterility is compromised. Prolonged periods of normothermic perfusion in the presence of antibiotics increase the risks of antibiotic resistant infections. It should, however, be noted that the authors believe there is a place for relatively brief periods of normothermic perfusion for assessment of liver performance, *ex vivo* treatment of the organ to minimize reperfusion injuries and possibly for restoration of the metabolic status of the organ, prior to transplantation.

Conclusions

The results suggest that both temperature ranges tested may be suitable for liver support for up to 10h of oxygenated HMP. Temperature in the 4-6°C range is much easier to maintain, using ice, than the 12-14°C temperature range. Use of the higher temperature range would increase the complexity, weight, power requirements and cost of the liver perfusion device. Therefore, further device development will focus on maintaining 4-6°C, although our *ex vivo* perfusion results suggest that short excursions up to 12-14°C are not detrimental. Further research is needed to evaluate the potential benefits of inhibiting ischemic reperfusion injury during longer periods of HMP prior to preclinical liver transplant studies.

Authors' contributions

KGMB was responsible for conception, design, funding, execution, interpretation and drafting the manuscript of this study. CYL provided advice on perfusion methods, assays, interpretation of results and revising the manuscript. EDG assisted in acquisition of data, analysis, manuscript preparation and revision. ZC and LKF assisted in performance of experiments including perfusate preparation, optimization of blood perfusate and development and performance of perfusate analyses. LHC assisted in all aspects of this study mentioned above and interpretation of data. All authors have had the opportunity to provide manuscript revisions and they have approved the final version.

Disclosure Statement

EDG, ZC, LKF and LHC are employees of the company, CYL is a paid consultant and KGMB is both an employee and owner of Cell & Tissue Systems, Inc.

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