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Extracorporeal Liver Perfusion System for Artificial Liver Support Across a Membrane

Jai Bikhchandani, M.R.C.S.,*[,1] Matthew Metcalfe, F.R.C.S.,* Severine Illouz, Ph.D.,* Florian Puls, F.R.C.Path.,† and Ashley Dennison, F.R.C.S.*

*Department of Hepatobiliary Pancreatic Surgery, University Hospitals of Leicester, Leicester, United Kingdom; and †Department of Pathology, University Hospitals of Leicester, Leicester, United Kingdom

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Background. **An extracorporeal porcine liver perfusion (ECPLP) system circumvents the limitations of hepatocyte based bio-artificial liver, but its clinical application has been limited so far due to the potential risk of transmission of porcine endogenous retroviruses. The aim of this study was to develop an ECPLP model that can provide artificial hepatic support across a semi-permeable membrane, which has the potential to block porcine viruses due to its pore size.**

Materials and Methods. **Livers from white landrace pigs were perfused with normothermic oxygenated blood using Medtronic BP560 centrifugal pump (Medtronic, Inc., Minneapolis. MN). This ECPLP system was used to support a "surrogate" patient across the filter Evaclio-EC4A. Function of liver was measured by indocyanine green retention at 15 min (ICGR15). Clearance of galactose, ammonia, and para-aminobenzoic acid infused into the "surrogate" patient circulation was calculated to assess liver support across the membrane. The study was designed as test ($n = 15$) *versus* control ($n = 5$), with control experiments having no liver in the circuit.**

Results. **For the test experiments, we perfused 15 livers with mean hepatic artery pressure of 87 mm Hg and flows of 1.2 L/min. ICGR15 in test experiments was 11%. Ammonia clearance was 945 mg/min/kg, galactose metabolic rate was 111.7 mg/min/Kg, and the hippurate ratio was 91% in the test. In contrast, the control experiments did not show any significant change in the concentration of any of these compounds.**

Conclusion. **Our ECPLP model was able to provide hepatic support in an experimental setting across a hollow fiber filter. Further work on an anhepatic animal is needed prior to application in human trials.**

Key Words: **extracorporeal; liver perfusion; bio-artificial liver; porcine; acute liver failure; porcine retroviruses.**

INTRODUCTION

Liver transplantation is the only available successful treatment of acute liver failure (ALF) with survival rates of up to 85% [1]. The clinical progression in fulminant hepatic failure is so rapid that these patients cannot wait on the transplant lists and therefore may face a very high mortality without a transplant. However, transient hepatic support can bridge these patients to safety with the end result being either availability of donor or regeneration of native liver. Support therapies based on non-biological adsorbent systems or biological devices are a subject of intense research. A bio-artificial liver (BAL) requires a large sustainable bio-mass of hepatocytes for which advanced and expensive tissue culture techniques are essential [2–5], making its widespread use difficult. BAL has also not shown any survival benefit so far in trials involving ALF patients [6–9]. Clinical application of isolated perfused liver for hepatic support is an alternative, especially with the recent progresses made in pediatric cardiopulmonary bypass technology. Some authors have reported on the early experience of using extracorporeal porcine liver perfusion (ECPLP) system for ALF patients [10–13]. The major obstacle in the further development of these systems is potential transmission

[1] To whom correspondence and reprint requests should be addressed at Department of Hepatobiliary Pancreatic Surgery, University Hospitals of Leicester, Gwendolen Road, Leicester LE54PW, United Kingdom. E-mail: jaibikhchandani@creighton.edu or jai_9c@yahoo.com.

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of porcine endogenous retroviruses (PERVs) into the human circulation. This problem can be solved by integrating a semi-permeable membrane in the ECPLP system that would inhibit the transfer of viruses while still allowing the free movement of albumin and hepatocyte growth factors. The current study was designed with the aim to set up such an ECPLP system and test its efficacy in an experimental setting.

MATERIALS AND METHODS

Porcine livers were retrieved from 50–60 Kg white landrace pigs at the abattoir. All animals used for the purpose of this research were treated as per "The Animal Welfare Act 2006, United Kingdom" that encompasses, among other things, a suitable environment, suitable diet, and ability to exhibit suitable behavior. The warm ischemia time was minimized and the preservative solution Soltran (Baxter Healthcare Ltd., Berkshire, UK) was infused *via* the portal vein. The isolated liver was then stored at –20°C till the start of normothermic perfusion. Autologous porcine blood, diluted (1:1) with Ringer's intravenous solution (Baxter Healthcare Ltd.), warmed at 39°C and oxygenated (95% O_2 and 5% CO_2), was used for perfusion. Table 1 lists the various substances infused to optimize the perfusate. A metallic chamber with a perforated metallic sheet was used for placing the organ during perfusion to allow drainage and collection of ascitic fluid secreted by the surface of perfused liver. The circuit designed for perfusing liver was a modified version of the one used by previous authors [13–17] (Fig. 1). It consisted of a centrifugal pump (Medtronic Inc., Minneapolis, MN), pump speed controller (Bio-console 560; Medtronic, Inc.), motor head (Medtronic external drive unit 540-T; Medtronic, Inc), oxygenator (Medtronic MinimaxTM 138, hollow fiber oxygenator), water bath (GD120; Grants Instruments Ltd., Cambridge, UK), soft shell reservoir (Medtronic MVR800), custom built glass reservoir, pressure transducer and pediatric flow transducer (Medtronic Bioprobe TX50P). The centrifugal pump controlled the hepatic artery pressures strictly between 80 and 100 mm Hg. The portal venous blood flow was gravity dependent. Flow through the hepatic artery *versus* portal vein was titrated to a ratio of 1:4 to mimic physiologic conditions. In order to test our ECPLP system in an experimental setting, a second circuit labeled as "surrogate patient circuit" was used. This comprised of a reservoir containing porcine blood with high levels of ammonia (analogous to a patient in ALF) circulated using a roller pump (Watson Marlow 505S; Watson-Marlow Pumps Group, Falmouth, Cornwall, England). The two circuits were linked in such a way that the circulating blood on either side can exchange substances by diffusion across the hollow fiber filter, Evaclio EC4A (Kuraray Medical Inc., Tokyo, Japan), acting as a semi-permeable membrane. The filter is made up of ethylene-vinyl alcohol copolymer, has a surface area of 2.0 m^2, and a pore size of 30 nm. Evaclio EC4A has sieving coefficients of approximately 0.82 for human albumin, 0.55 for human IgG, and 0.14 for fibrinogens [18]. This membrane would allow free exchange of molecules up to the size of 80 kDa but block permeation of larger molecules like immunoglobulins (150–900 kDa) and porcine retroviruses (4000–6000 kDa). The "liver perfusate" was circulated through the extra-luminal compartment of this filter while the "surrogate patient blood" through the intra-luminal compartment of the filter (Fig. 1).

Biopsies (0.5 cm × 0.5 cm) were taken from the right and left lobes of liver before the start of perfusion and at the end of 6 h of perfusion. The tissue was immediately fixed in formal saline (10%). Blood samples were drawn from the liver perfusate at 0, 3, and 6 h of perfusion for hematological and bio-chemical assessment. Perfusate specimens were also taken at 1, 3, and 6 h, to measure oxygen extraction capability of the liver by applying Fick's principle and using the equation below:

$$\text{O2 consumed in mL/min/g of hepatic tissue} = \frac{(\text{O2 content in hepatic artery} - \text{O2 content in IVC}) \times \text{total blood flow}}{\text{Weight of liver in grams}}$$

$$\text{O2 content} = 1.39\ (\text{Hb in g/dL})\ (\text{O2 sat as \%}) + 0.0031(\text{pO2})$$

IVC : inferior vena cava

Functional capacity of the isolated perfused liver was objectively measured using ICGR15 test, i.e., Indocyanine green (Pulsion Medical Systems, Middlesex, UK) 15 min retention. ICG is a water soluble tricarbocyanine dye which binds to intravascular proteins mainly albumin, taken up exclusively by the hepatic parenchymal cells and secreted unchanged into bile. Clearance of ICG can be measured by spectrophotometric analysis to assess the hepatic blood flow and hepatocyte function [19, 20].

ECPLP system was run for 6 h during which the test substances were infused into the "surrogate patient" circulation at regular intervals. In a functioning liver, circulating ammonia should be metabolized by the urea cycle. A bolus of ammonium chloride 1000 mg (NH4Cl; Sigma Aldrich, Dorset, UK) was therefore injected at the start of every hour of perfusion after the first 60 min of stabilization of extracorporeal circulation. Deproteinized blood samples from the surrogate patient were analyzed using ammonia test kit (Boehringer Mannheim/R-Biopharm, Roche, Berks, UK) to calculate ammonia clearance by a method previously described [21]. Galactose elimination reflects the capacity of hepatic microsomal enzyme system and

TABLE 1

Substances Infused to Optimize the Perfusate During ECLP

One time boluses	
Flolan	20 mL (500 μg in 50 mL of diluent, diluted with 200 mL of N/S)
Sodium bicarbonate (8.4%)	40 mL
Cefuroxime	750 mg in 5 mL of water
Calcium chloride	10 mL
Scheduled boluses	
Heparin	1000 units/h
Clinimix (aminoacids only)	15 mL/h
Continuous infusions	
Sodium bicarbonate (8.4%)	40 mL/h
Flolan (prostaglandin E1)	20 mL/h (500 μg in 50 mL of diluent, diluted with 200 mL of N/S)
Sodium taurocholate	10 mL/h (2% wt/vol, 2 g in 100 mL of N/S) i.e., 200 mg/h
Insulin	50 U/h

N/S = 0.9% normal saline.

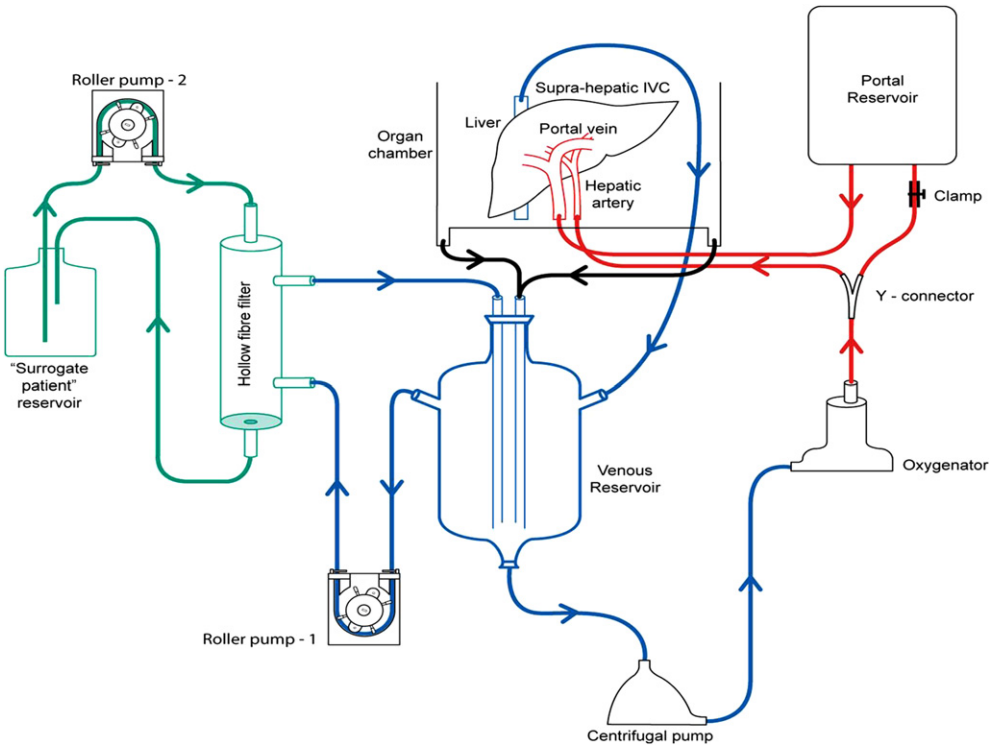

FIG. 1. ECPLP circuit.

has been found to be prognostic in chronic liver disease in humans [22]. Tygstrup proposed elimination kinetics of galactose in 1966 [23]. Galactose is phosphorylated into galactose-1-phosphate by galactokinase in hepatocyte cytoplasm. Galactose elimination Vmax is the elimination rate of galactose by the enzyme galactokinase, which is a measure of functional hepatic mass. Galactose (30 g, anhydrous, C6H12O6; Sigma-Aldrich, Dorset, UK) was infused at "hour 2" and "hour 6" of perfusion. Lactose D-galactose kit (Boehringer Mannheim/R-biopharm, Roche) was used for galactose assay, the methodology of which has been described before [21]. Para-aminobenzoic (PABA) is metabolized by phase II conjugation reactions in the liver to para-amino-hippuric acid (PAHA), para-acetamidobenzoic acid (PAABA) and para-acetamido-hippuric acid (PAAHA) [24]. Glycine conjugation and hepatic acetylation of PABA is significantly impaired in liver failure. Quantitative measurement of hippurates *versus* parent compound PABA was done to calculate the hippurate ratio as follows:

$$\frac{[\text{PAHA (M)} + \text{PAAHA (M)}]}{[\text{PAHA (M)} + \text{PAAHA (M)} + \text{PAABA (M)} + \text{PABA (M)}]} \times 100\%$$

Two hundred mg of PABA (4-aminobenzoic acid, Sigma-Aldrich) was infused into the surrogate patient circulation. Serum PABA and its metabolites were measured according to the method described by Furuya *et al.* using solid-phase extraction [25].

The study was designed with "controls" to confirm that the hollow fiber filter was not a confounding factor. In the "control" experiments; the surrogate patient circuit was linked *via* the filter to the extracorporeal perfusion circuit with no liver and the clearance of ammonia, galactose and PABA was measured. A total of 15 "Test" experiments and 5 "Control" experiments were performed. Reperfusion injury to the liver parenchyma was assessed by measuring hepatic enzyme levels prior to start of perfusion and then after 3 and 6 h. Sections from the left and right lobe of the organ, pre-perfusion and post-perfusion were reviewed by a pathologist for hydropic hepatocytes, fibrin thrombi, hemorrhage, edema, distended lymphatics, sinusoidal dilatation; infiltration by neutrophils, lymphocytes and Kupffer cells, and percentage necrosis in the hepatic lobules.

RESULTS

Organ retrieval from abattoir animals involved a period of warm ischemia (5.4 ± 1 min, range 4–7.8) before Soltran solution (Baxter Healthcare Ltd.) was infused through the portal vein. Extracorporeal perfusion required back-table preparation of porcine liver and priming of the circuit which contributed to the cold ischemia time of 173 (SD 11, range 150–185) min with the organ stored at –20°C. The various parameters used in the study to test the functioning and viability of extracorporeally perfused liver were color of the liver, secretion of ascitic fluid by the organ surface, bile excretion, oxygen consumption, urea synthesis and ICGR15 test. A comparison of hemodynamics in 15 tests *versus* 5 controls is presented in Table 2. Figure 2 show that perfusion pressures ($n = 15$) were tightly regulated to generate flows that varied depending on the vascular resistance of the *ex vivo* organ (Fig. 3). Mean oxygen extraction ranged between 28 to 33 μL/min/g of hepatic tissue during the course of perfusion. Porcine livers produced an average of 7 mL of bile per hour. There was a 312% increase in the baseline level of urea in the perfusate suggestive of urea synthesis in the liver over the 6 h of

TABLE 2

Extracorporeal Perfusion Parameters: Test *Versus* Control

	Test ($n = 15$) mean ± SD	Control ($n = 5$) mean ± SD	P value
Hepatic artery pressure (mm Hg)	87.3 ± 3.5	81.4 ± 3.4	NS
Portal vein pressure (mm Hg)	26.6 ± 3.6	18.2 ± 2.4	NS
Hepatic artery flow (mL/min)	277.7 ± 33.6	264 ± 20.7	NS
Portal vein flow (mL/min)	967.3 ± 178.9	910 ± 88.6	NS
Total flow (mL/min)	1244.7 ± 185.4	1214 ± 77	NS
Bile produced	7 ± 3.3	0	Highly significant
ICGR15 test (%)	11.4 ± 4.6	96.4 ± 2	0.001

NS = not significant.

perfusion. ICGR15 test was done prior to linking up the ECPLP system across the filter with only 11% (mean) of the infused dye remaining in the system (Fig. 4).

The ECPLP system was tested for its bio-hepatic capacity by calculating the ammonia clearance, galactose elimination Vmax, and hippurate ratio in the surrogate patient circulation. The comparison of results in test *versus* control group in Table 3 clearly show that the functional capacity of ECPLP system was dependent on the presence of a perfused liver and the hollow fiber filter simply played the role of a semi-permeable membrane allowing diffusion. Ammonia clearance was measured at every hour of perfusion. In the test experiments, this followed first order kinetics as reported previously by Uesugi *et al.* [21]. The percentage of ammonium chloride (1000 mg) cleared within 15 min of injection by the ECPLP system ranged from 67% to 80.6%. Mean ammonia clearance (mL/min) was also calculated. There was some variability seen at different time intervals of perfusion but the clearance was always greater than 850 mL/min (Fig. 5). Galactose elimination analysis tests were performed at h 2 and h 6 of perfusion. Metabolism of galactose in the test experiments followed zero order kinetics. Figure 6 demonstrates that the metabolic capacity of liver remained stable over 6 h. Hippurate ratio measures the efficacy of hepatic detoxification of PABA. Figure 7 shows that the hippurate ratio was between 74% and 97% during the 15 test experiments (90.3% ± 7.3 %). More than 90% of infused PABA was converted into glycine conjugated metabolites within 30 min of infusion. In our research, we studied the function of isolated liver with no other associated viscera. Therefore, there is no question of any extrahepatic metabolism of PABA injected in the system and any hippurated metabolites of PABA would indicate hepatocyte function.

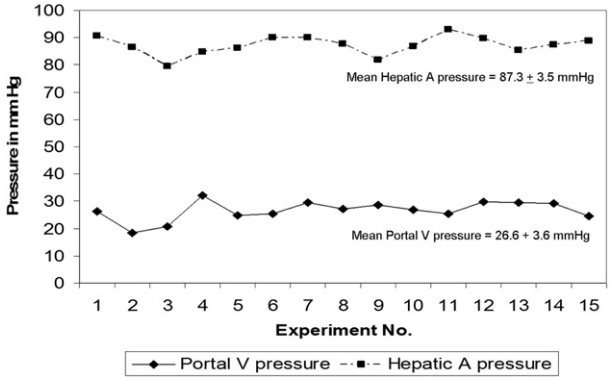

FIG. 2. Extracorporeal liver perfusion pressures.

Hepatocytes sustained injury during warm, cold ischemia and subsequently during extracorporeal perfusion. This accounted for a 9-fold increase in the level of alanine transaminase at h 3 from baseline. The enzyme levels at the third and sixth hour of perfusion remained essentially similar; an evidence that extracorporeal perfusion related injury was minimal. On histologic assessment, the median hepatic lobular necrosis seen in the post-perfusion liver sections was 20% (Fig. 8).

DISCUSSION

Acute liver failure (ALF) is a life threatening illness without a liver transplant. A patient in ALF while waiting on the transplant list may succumb to the disease before the organ becomes available [26]. Therefore, a temporary liver support device to tide over the interim period could be life saving. ALF patients are relatively younger than those in chronic liver failure and the pre-morbid state of the organ is physiologically normal. Hence, there is a potential of regeneration [27], but only if adequate hepatic support needed for survival is provided during the recovery period. Several non-biological systems have been proposed, which work on one or the other filtration principles, but the success of these systems is doubtful because they only replace detoxifying functions of the liver [28]. The metabolic and synthetic functions of liver still need to be addressed. An increasing number of bio-artificial liver devices (BAL) have been developed. BAL relies on the functionality of hepatocytes (xenogeneic or human in

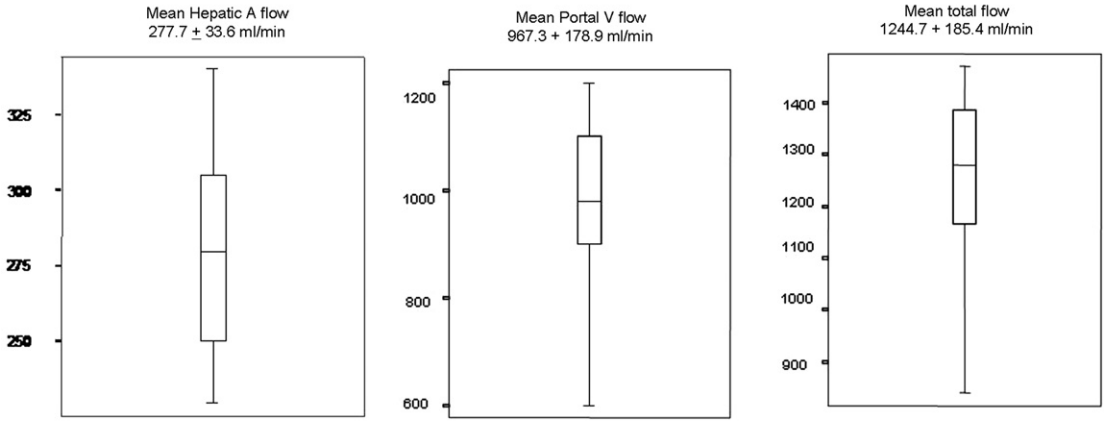

FIG. 3. Box plot of mean blood flow to the liver during test experiments (n = 15).

origin) to support the patient. Hepatic encephalopathy occurs when liver function falls below 25%–30% of normal. Therefore, it can be said that about 200 to 400 g of hepatocytes are needed to provide effective liver support [29]. Advanced tissue culture techniques are essential to produce and maintain viability of hepatocytes at such a high cell density. Culture in three-dimensional matrices, or co-culturing with non-parenchymal cells—Kupffer, sinusoidal, and Ito cells, or continuous perfusion feeding of hepatocytes prolong their function and survival [2–5]. This technology may not be readily available and may be very expensive. If we rethink, bio-reactors are striving to achieve the sinusoidal architecture of hepatic tissue. So, the question that arises is—why not simply use the whole organ for liver support? This would be much cheaper and cost effective. Moreover, the clinical efficacy of BAL is still questionable. Several different BAL devices have been applied clinically but none of these has shown significant effect on survival in controlled clinical trials in ALF patients [6–9].

Advancements in extracorporeal membrane oxygenation and cardiopulmonary bypass technology have enabled stable prolonged *ex vivo* liver perfusion optimal for clinical application [17]. An ideal organ based system should derive support from a human liver. Fox *et al.* [10] have used human livers that were rejected for clinical transplantation to treat a small series of patients with some success. In the current age of utilizing marginal as well as non-heart-beating donors for transplant, it is not practical to use human livers anymore. Hence, most of the clinical studies have used porcine livers for whole organ support [11–13]. However, reports of porcine endogenous retroviruses (PERV) infecting human cells are now the main deterrent in the clinical application of ECPLP as a liver support system [30, 31]. Direct exposure of patients' blood to porcine tissue, though practiced in the past, does pose a theoretical

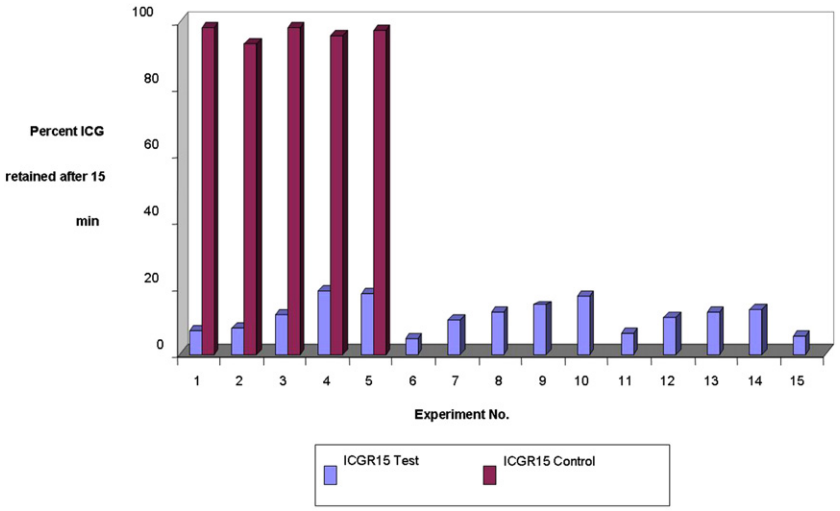

FIG. 4. ICGR15: Test versus Control.

TABLE 3

Bio-hepatic Capacity of ECPLP System

	Test ($n = 15$) mean ± SD (range)	Control ($n = 5$) mean ± SD (range)	P value
Galactose elimination Vmax (mg/min/Kg)*			
Hour 2	116 ± 28.1 (64.9 – 180.5)	6.5 ± 5.4 (1.1 – 14)	<0.001
Hour 6	106.8 ± 35.3 (40.1 – 169.5)	6.7 ± 7.1 (1.4 – 19.2)	
Ammonia cleared in 15 min (%)†			
Hour 2	80.6 ± 11.8 (57.6 – 97)	2.7 ± 12.1 (–9.9 – 21.6)	<0.001
Hour 3	75.6 ± 17.7 (51.5 – 91.6)	2.4 ± 6.6 (–5.8 – 9.6)	
Hour 4	72.2 ± 17.1 (43.5 – 94.7)	−0.6 ± 4.3 (–7.3 – 4.4)	
Hour 5	67 ± 20.1 (27.8 – 92.8)	−1.4 ± 3.1 (–4.7 – 2.9)	
Hour 6	70.7 ± 15.5 (29.1 – 87.8)	−2 ± 4.1 (–8.6 – 1.9)	
Hippurate ratio (%)‡			
Hour 5	90.7 ± 6.58 (74.3 – 96.9)	0	Highly significant

*Galactose: 30 g dissolved in 60 mL of water.
†Ammonia: 1000 mg NH_4Cl in 10 mL of water.
‡PABA: 200 mg of PABA dissolved in 40 mL of water at 25°C.

threat for transmission of xenogeneic viruses into the human circulation. Application of dialysis membrane technology in ECPLP models is needed. Incorporation of a hollow fiber filter with an effective pore size of 80–100 kDa would allow diffusion of toxins and metabolites across the membrane while providing protection against PERV [32]. The question is whether artificial hepatic function can be provided *via* such a membrane. Our study was designed as a pilot study to answer the above question using an ECPLP model in an experimental setting.

Liver perfusion based on abattoir organs was run for at least 6 h, a duration that was adequate to answer the question of interest. Authors have already shown ECPLP to work for up to 72 h by retrieving organs from live anaesthetised pigs [17, 33]. Our work emphasized the opinion of other authors [15–17] that an "oscillating pressure chamber for liver" is not essential for venous outflow from an extracorporeally perfused liver. In our study, the mean perfusion pressure in the hepatic artery was 87.3 ± 3.5, range 79.5–93.1 mmHg. This was comparable to the pressures reported by Butler *et al.* while perfusing *ex vivo* livers, i.e., 90.3 ± 9.5 mmHg [17]. Flows achieved through hepatic artery were also comparable with other authors at a mean of 0.28 ± 0.03 L/min (our study) *versus* 0.24 ± 0.18 L/min (Butler *et al.* [17]). Mean total flow of 1.2 ± 0.2 L/min was achieved in our extracorporeally perfused livers.

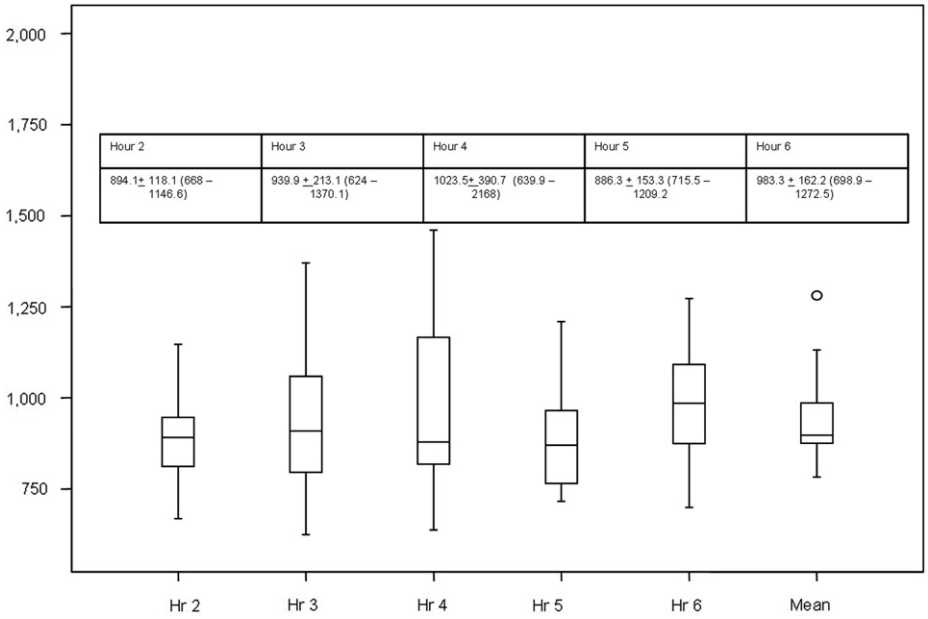

FIG. 5. Box plot of mean ammonia clearance in ml/min at hourly intervals (n = 15).

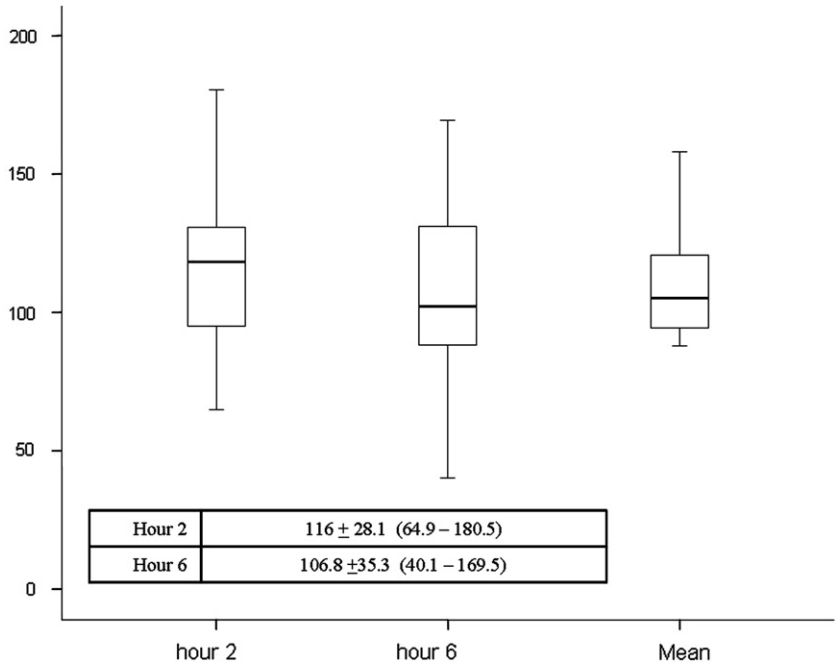

FIG. 6. Box plot depicting the stability of Galactose Vmax (n = 15) during perfusion.

Our ECPLP model was able to generate about 7 mL of bile per hour, a volume similar to that published in other studies [34, 35]. The optimum perfusion of any tissue is shown by its capability to extract oxygen. The role of oxygen extraction as a measure of adequate extracorporeal liver perfusion has been highlighted by a number of researchers [17, 36, 37]. The mean oxygen consumption reported by Mrus *et al.* was 9 μL/g of liver/min [36]. In our study, the mean oxygen extraction of *ex vivo* perfused liver ranged from 28 to 33 μL/min/g over the 6 h of extracorporeal perfusion. ICGR15 was selected as another objective indicator of function of isolated perfused liver. Only about 11% (mean) of ICG was retained in the perfusate after 15 min. Borie *et al.* reports an ICGR15 of around 30%–35% in the isolated perfused livers [37]. Normal ICGR15 in healthy human volunteers range between 3.5%–10 % [38]. Urea synthesis is an essential function of liver for the disposal of body's nitrogen and maintenance of acid base balance. Various authors have used synthesis of urea as a parameter to assess the synthetic capacity of isolated perfused liver [17, 37, 39]. Butler *et al.* have shown the mean urea level to rise from 2.9 mmol/L to 45.4 mmol/L after a period of 72 h of extracorporeal perfusion [17]. In our study, urea levels increased from 2.7 to 11 mmol/L over 6 h.

Selection of the correct semi-permeable membrane was vital to our study. The membrane must be permeable to albumin (66 kDa) for clearance of bilirubin and other toxins bound to it. It must also allow diffusion of the hepatocyte inhibitory factor (25 kDa) so that it gets cleared from the circulation of a patient in ALF. On the other hand, the membrane should inhibit free movement of the smallest porcine endogenous retrovirus (120 kDa) and hepatocyte growth factor (84 kDa) across it. So, the pore size chosen should be between 50 and 100 kDa [40]. The above

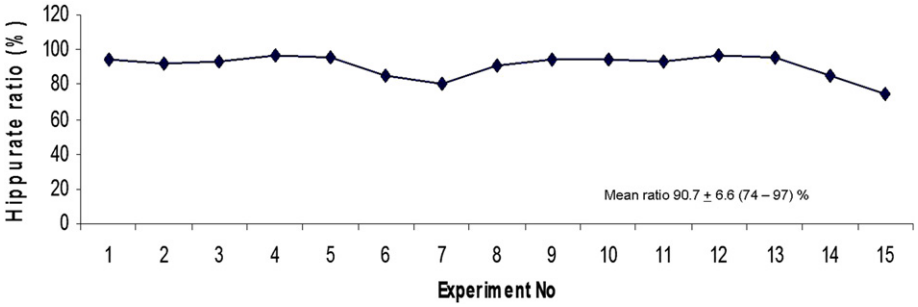

FIG. 7. Hippurate ratio: Test experiments (n = 15).

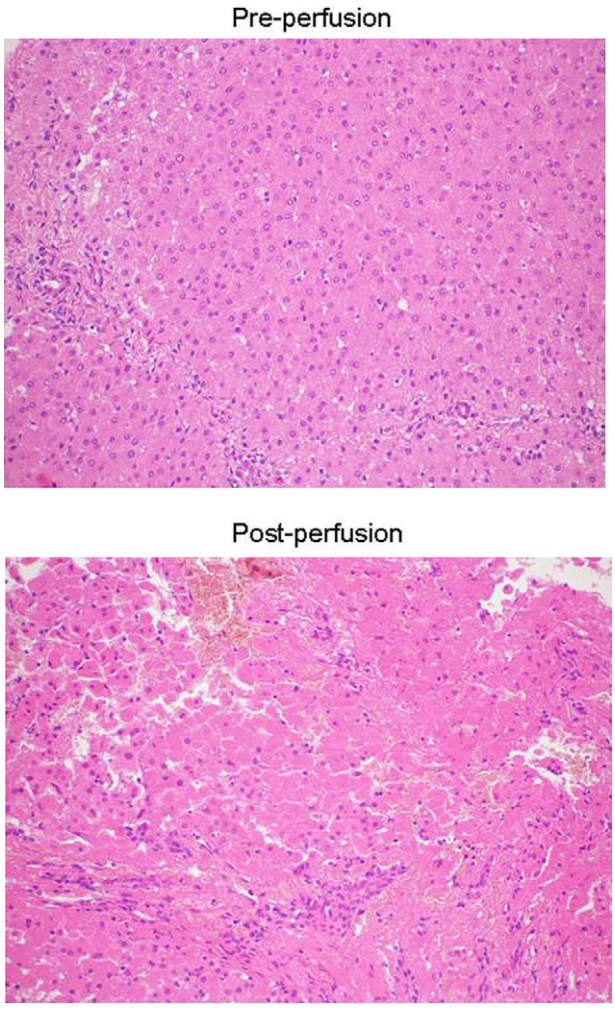

FIG. 8. Sections from left lobe of liver in ECPLP test no 11.

properties were found in Evaclio EC4A. Sieving coefficient of Evacure EC4A for albumin is greater than 0.6, i.e., more than 60% of albumin is able to cross this membrane when the blood is circulated through the filter.

After linking the "surrogate patient" to the ECPLP *via* Evaclio EC4A, clearance of ammonia, galactose, and PABA was measured at sequential time intervals to demonstrate the functioning of the ECPLP system across the membrane. The mean Galactose Vmax in our set of experiments was 111.7 + 22.8 (87.8–158.0) mg/min/kg of liver. Vmax was 116.6 + 28.1 in h 2 and 106.8 + 35.3 in h 6 of perfusion. Uesugi *et al.* [21] reported galactose elimination capacity of alloperfused porcine livers to be 109 + 12 mg/min/kg of liver in the first hour and 92 + 9 mg/min/kg of liver in the ninth hour of perfusion. Imber *et al.* [34] have shown the Galactose Vmax to be 76 mg/min/kg in an extracorporeally perfused porcine liver. Comparison with similar work by other authors shows that there was no compromise in the metabolic function of liver across the membrane. Clearance of ammonia from the circulation is an essential function to replace in a patient with failing liver. In our study, 73.2% (mean) of injected ammonium chloride was cleared within 15 min. Borie *et al.* reported that after infusing large doses (500-1000 mg) of ammonium citrate during extracorporeal liver perfusion, 21 + 6% of the injected dose remained after 45 min [37]. The mean ammonia clearance in our ECPLP system ranged from 886.3 mL/min to 1023.5 mL/min over the six hour duration of perfusion; with the system being most efficient in clearing ammonia after 3 h of perfusion. This seems logical as the *ex vivo* liver attains a stable state after the first few hours. Para-amino-benzoic Acid (PABA) has been described as an ideal probe drug to quantify hepatic function [41]. The mean hippurate ratio in our study was 90%, suggesting adequate bio-hepatic support by the ECLP to the surrogate patient. Furuya *et al.* [25] reported that the hippurate ratio in healthy volunteers was 60%.

Our study was limited by the fact that the liver for ECPLP was retrieved from the abattoir animals and it was impossible to completely eliminate warm ischemia prior to perfusion. Clinical application of any sort on porcine-based ECLP would mandate organ harvest on anaesthetised, specially bred animals meant for clinical use. The primary objective of the current study was to design an extracorporeal porcine liver-based perfusion system, which could reliably provide artificial hepatic function across a hollow fiber filter acting as a semi-permeable membrane. Specific testing was not performed as part of this study to prove that the porcine endogenous retroviruses do not transmit across this hollow fiber filter into the surrogate patient circulation. Further studies using polymerase chain reaction (PCR) assays would be needed to confirm this.

CONCLUSIONS

Clinical application of ECPLP for liver support received a setback with the reports of PERV infecting human cells in culture. The only way to advance research in this direction is to find a way of eliminating the risk of PERV transmission in humans. Artificial bioreactors based on hepatocytes have used membrane technology for the above reasons. We, therefore, attempted to apply the same in ECPLP. Our results on galactose elimination, ammonia clearance, and PABA metabolism proved with reasonable confidence that our ECPLP system worked well across the semi-permeable membrane Evaclio EC4A, which has the potential to block PERVs. Reliable results were achieved to proceed with future work on ECPLP that would involve testing

the efficacy of this system in an anhepatic animal prior to clinical use.

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