Short communication

# Study of porcine hepatocyte-entrapped bioartificial liver in surgery-induced fulminant hepatic failure rabbits

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## ARTICLE INFO

## ABSTRACT

ICP is an important cause of mortality in fulminant hepatic failure. The aim of this study was to prolonged survival in surgery-induced FHF rabbit with BAL. Four hours after induction of FHF, rabbits were connected to a 4-h whole blood perfusion through the BAL containing $1.2 \times 10^9$ fresh isolated porcine hepatocytes (estimated 30% of liver volume of rabbit) via an arterial-venous shunt. Survival times in control group, sham-BAL group and BAL group were $14.4 \pm 4.4$ h, $12.6 \pm 2.6$ h and $22.6 \pm 5.2$ h, respectively (BAL vs. control, $p = 0.026$; BAL vs. sham-BAL, $p = 0.006$). BAL group remain ICP level less than 10 mmHg for near 13 h after FHF induction while 19 and 15 mmHg were observed in control and sham-BAL groups, respectively. BAL containing porcine hepatocytes significantly prolonged survival time by delayed increase of intracranial pressure compared with the control and sham-BAL groups.

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## 1. Introduction

Fulminant hepatic failure (FHF) is a serious and fast progressing disease with high mortality (60–90%), despite the fact that the liver is an organ with enormous potential for regeneration. Due to a donor shortage, development of a hybrid artificial liver is important required. Bioartificial liver (BAL) devices consist of isolated hepatocytes with extracorporeal circulation systems [1–4]. Clinical research on BAL in patients with FHF has been increasing over the last decade. Many researchers have indicated the relation or correlation between the expression of the native liver functions and/or cell morphology in cultured primary hepatocytes (or hepatoma cells). Before BAL can be applied in human therapy, a suitable animal model is needed to test these systems in terms of safety and feasibility. However, despite the complications of FHF, several models have been created to obtain an acceptable large animal model (pigs and dogs) to study the treatment of FHF [5–10], but there are only a limited number of experimental studies that treat FHF rabbits with BAL containing porcine hepatocytes. Due to the high cost of studies on large animals, rat or rabbit models are especially needed for bioartificial liver tests [11–14].

Ijima et al. used a packed-bed module with spheroids formed by adult rat hepatocytes embedded polyurethane foam (PUF) as a hybrid artificial liver and developed an extracorporeal circulation line for recovery of a hepatic failure rat induced by D-galactosamine (D-Gal). Their results showed blood ammonia concentrations in hepatic failure rats were suppressed below the hepatic coma level by the artificial liver support system and about 80% of these rats can be recovered from hepatic failure [13].

Wang et al. evaluated extracorporeal bioartificial liver support system (EBLSS) consisting $1 \times 10^8$ of spheroidal primary hepatocytes which isolated from 4-month-old human fetal liver to treat D-Gal-induced FHF rabbits [15]. Their results showed increases in serum AST, but TB and Cr were restrained effectively in cell-supported animals. They further suggested that the surgically induced FHF rabbits is reproducible and provides measurable clinical and biological features.

Earlier we developed a model of surgically induced FHF in rabbits combining resection of the three anterior lobes (74.12% of liver mass) with ligation of the pedicle of the right lateral lobes (20.22% of liver mass), resulting in liver necrosis; the remnant quadrate lobes (5.67% of liver mass) were left intact [14]. In our previous studies, the isolation and culture of human hepatocytes and fresh isolated

porcine hepatocytes entrapped in a hollow fibers-based bioartificial liver device (HF-BAL) were established [16,17]. The aim of this study was to evaluate the ICP and survival in surgery-induced FHF rabbit with HF-BAL system. ICP changes may acts as an indicator because it showed higher correlation with survival time than other biochemistry data. The works provide a good candidate for the rapid evaluation of BAL system in animal model.

## 2. Materials and methods

### 2.1. Animal preparation

Adult male, New Zealand white (NZW) rabbits, weighing 2.4–3.1 kg ($n = 15$), received water and pellet feed ad libitum and were kept in cages at 21 °C on a 12-h light/12-h dark cycle. Male pigs weighing between 10 and 14 kg were used for isolation of hepatocytes.

### 2.2. Induction of fulminant hepatic failure

A surgical model of FHF in the rabbits was prepared according to our previous study [14]. In brief, all surgical preparations were performed under sterile surgical techniques and general anesthesia. Intravenous lines were set via internal jugular vein and saline (20 ml/kg) via a right ear marginal vein with infusion of saline. Body temperature monitored by rectal probe in FHF animals was maintained by heating pads and lamp. The sequences of the procedure were central line operation, induction of FHF followed by the burr-hole procedure. The creation of FHF included resection of three anterior lobes, ischemic change of right lateral lobe and intact quadrate lobe. Following the hepatic procedure, a burr hole at right parietal skull bone was created for inserting a Codman MicroSencor catheter (Codman MicroSencor, Johnson & Johnson, MA, USA) to measure intracranial pressure (ICP).

### 2.3. Hepatocyte isolation

Hepatocytes were harvested from white male pigs ($n = 5$) weighing 10–14 kg ($11.9 \pm 1.8$ kg) using a two step in situ collagenase perfusion technique modified from the method described by Seglen [18] and Nyberg [19]. Briefly, animals were anesthetized intravenously with Ketamine (1 ml/kg) and Rompum (1 ml/kg). After portal vein cannulation, in vivo perfusion (100–300 ml/min × 10 min) was performed using a calcium-free hydroxylethylpiperazine ethane sulfonic acid (HEPES)-buffered solution. The resected liver was transferred into a laminar flow before perfusion (100–300 ml/min × 20 min) in a harvest pot containing a second HEPES-buffered solution containing 0.05% Type II collagenase. The soft liver was rubbed and filtrated through a stainless steel filter (pore size 104 μm). The filtrate was centrifuged and washed twice using a COBE machine (COBE 2291$^{TM}$, cell processor) at $50 \times g$ for 2 min. Each pig harvest yielded from $1.01 \times 10^{10}$ to $2.7 \times 10^{10}$ ($1.56 \pm 0.7 \times 10^{10}$) hepatocytes. Cell counts and viability were measured by trypan blue exclusion. Only hepatocyte suspensions with viability >85% were used.

### 2.4. Assembly of bioartificial liver system

The hollow fibers in the cartridge were made of mixed cellulose ester (ME) with a nominal molecular weight cut-off (MWCO) of 0.2 μm, and their inner diameter and surface area were 0.6 mm and 460 cm$^2$, respectively. The total volume of the inner space of the hollow fibers was 5 ml. The volume of the shell space between the hollow fibers and the cartridge housing was about 22 ml. Fresh porcine hepatocytes were suspended at a concentration of $5.4 \times 10^7$/ml in 10% serum containing William's E culture medium and seeded immediately by syringing into the shell space of the hollow fibers cartridge at a density of $1.2 \times 10^9$ hepatocytes per bioreactor [17]. Both ends of the shell space of the hollow fibers cartridge were closed using two sterilized plastic caps and was then placed in a closed circuit (total volume 4.2 ml). Before connecting to FHF rabbits, 500 ml of medium was pumped through the flow circuit at a flow rate of 6 ml/min and sterilized air at a rate of 60 ml/min was diffused into the gas compartment via silicone tube. Dissolved oxygen and oxygen uptake rate (OUR) were monitored off-line with a blood gas analyzer (Model 278, CIBA-Corning, Bayer Diagnostics Corp., Norwood, MA, USA). Arterial blood gas, alanine transaminase (ALT), aspartate transaminase (AST), ammonia, total bilirubin (TB), lactate dehydrogenase (LDH), prothrombin time (PT), sodium, and potassium levels were also measured using standard techniques.

### 2.5. BAL treatment

All animals were medically supported and divided into three groups: control group rabbits ($n = 5$) were given only standard medical support without BAL treatment; the BAL group ($n = 5$) rabbits were treated with BAL system inoculated with $1.2 \times 10^9$ fresh isolated porcine hepatocytes; the sham-BAL group ($n = 5$) rabbits were treated with BAL system but without cells. Six hours after induction of FHF, animals in the BAL and sham-BAL groups were connected to a 4-h whole blood perfusion at a rate of 6–10 ml/min through the BAL containing $1.2 \times 10^9$ fresh isolated porcine hepatocytes in BAL group or without hepatocyte in sham-BAL group via an AV shunt as illustrated in Fig. 1.

### 2.6. Statistical analysis

Data are reported as mean ± SD. Statistical analysis was performed using ANOVA and student's t-test. Probability values less than 0.05 were considered significant.

## 3. Results and discussion

### 3.1. BAL system on surgical-induced FHF rabbit

Liver failure is a very complex clinical condition that requires a combination of multi-organ investigations. Many artificial liver support systems based on viable cells, namely bioartificial liver, have been published worldwide. However, the lack of knowledge is still huge as the complex functions of the liver cannot be solved easily [20–23]. In fact, the multiple and complex functions of the liver can be replaced only by using biologic substrates (i.e., hepatocytes, liver slice) [24–27]. The goal of this study was to test HF BAL containing fresh isolated porcine hepatocytes on surgical-induced FHF rabbit model which as our reported previously [14].

In this study, fresh isolated porcine hepatocytes were loaded into the extra-capillary space of the hollow fiber bioreactor in which cells were allowed to suspend, aggregate and attach to the surface of hollow fibers. The biochemical data showed that the entrapped cells prolonged survival time in FHF rabbits with statistically significant and the vital signs were also remained stable in BAL supported FHF rabbits.

Basically, all rabbits tolerated the protocol of surgically induced FHF and recovered smoothly from anesthesia. Four hours after FHF induction, the BAL and sham-BAL group rabbits accepted 4 h of BAL treatment with or without porcine hepatocytes, respectively. As shown in Fig. 2, after creation of FHF, the mean survival times of the rabbits in the control group were $14.4 \pm 4.4$ h (8–18 h). In the sham-BAL group, all except one rabbit accepted only 3 h of BAL treatment due to the unstableness of the blood flow; the survival times were