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Randomized Trial of Spheroid Reservoir Bioartificial Liver in Porcine Model of Posthepatectomy Liver Failure

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Abstract

Acute liver failure (ALF) is a catastrophic condition that can occur after major liver resection. The aim of this study was to determine the effects of the spheroid reservoir bio-artificial liver (SRBAL) on survival, serum chemistry, and liver regeneration in posthepatectomy ALF pigs. Wild-type large white swine (20 kg–30 kg) underwent intracranial pressure (ICP) probe placement followed by 85% hepatectomy. Computed tomography (CT) volumetrics were performed to measure the extent of resection, and at 48 hours following hepatectomy to assess regeneration of the remnant liver. Animals were randomized into three groups based on treatment delivered 24–48 hours after hepatectomy: Group1—standard medical therapy (SMT, n = 6); Group2—SMT plus bio-artificial liver treatment using no hepatocytes (0 g, n = 6); and Group3—SMT plus SRBAL treatment using 200 g of primary porcine hepatocyte spheroids (200 g, n = 6). The primary endpoint was survival to 90 hours following hepatectomy. Death equivalent was defined as unresponsive grade 4 hepatic encephalopathy or ICP greater than 20 mmHg with clinical evidence of brain herniation. All animals in both (SMT and 0 g) control groups met the death equivalent before 51 hours following hepatectomy. Five of 6 animals in the 200-g group survived to 90 hours ($P < 0.01$). The mean ammonia, ICP, and international normalized ratio values were significantly lower in the 200-g group. CT volumetrics demonstrated increased volume regeneration at 48 hours following

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.30184/supinfo

hepatectomy in the 200-g group compared with the SMT ($P < 0.01$) and 0-g ($P < 0.01$) groups. Ki-67 staining showed increased positive staining at 48 hours following hepatectomy ($P < 0.01$).

Conclusion: The SRBAL improved survival, reduced ammonia, and accelerated liver regeneration in posthepatectomy ALF. Improved survival was associated with a neuroprotective benefit of SRBAL therapy. These favorable results warrant further clinical testing of the SRBAL.

Acute liver failure (ALF) is a devastating condition with up to 30%–50% mortality rate in developed countries despite advances in critical care and liver transplantation.⁽¹⁾ Some cases may be salvaged by vigorous medical treatment together with the liver's capacity for regeneration, but orthotopic liver transplantation remains the definitive therapy for medically refractory ALF.⁽²⁾ The liver's remarkable regenerative capacity has been recognized since ancient Greek mythology (i.e., Prometheus). Supportive therapies such as bio-artificial livers (BALs) are designed to provide a favorable milieu for regeneration by detoxifying waste molecules and providing synthetic function. These devices should bridge the patients until donor organs became available, or ideally, allow the patients to avoid liver transplantations altogether.

First-generation devices have undergone clinical trials but thus far have failed to demonstrate a survival benefit over controls in randomized clinical trials.^(3–5) Newer generation devices have been modified to increase cell numbers and improve detoxification in large animal ALF models.^(6–8) One such device is the spheroid reservoir bio-artificial liver (SRBAL), which showed a survival benefit and was associated with both a significant lowering of blood ammonia levels and a neuroprotective effect with reduced cerebral edema formation in a drug-induced ALF model.⁽⁹⁾ In addition to ammonia detoxification, a hypothesized benefit of BAL therapy is the promotion of endogenous liver regeneration. Human and animals with ALF treated by BAL have been shown to have increased expression of the regenerative marker Ki-67, along with increased hepatocyte growth factor levels and liver-specific gene expression.^(9–11) To further study this effect, we designed a randomized trial of SRBAL treatment in a porcine model of posthepatectomy ALF, in which regeneration of the remnant liver was quantified by computed tomography (CT) volumetrics, Ki-67 staining, and serum cytokine levels.

To date, many preclinical large animal trials have used drug or liver toxin-induced models of ALF because of their relatively high incidence in developed countries. However, surgical patients are also at risk of ALF after major hepatectomy. Even in the setting of advanced surgical techniques such as portal vein embolization⁽¹²⁾ and the ALPPS (associating liver partition and portal vein ligation for a staged hepatectomy) procedure,^(13,14) the incidence of posthepatectomy ALF can approach 10% of cases.^(15–18) Toxin-induced and posthepatectomy ALF do not have the same pathophysiology, and the results in toxin-induced ALF studies may not apply to these surgical patients. This study investigates the effects of SRBAL in posthepatectomy ALF pigs with the goal to further expand management options in this patient population.

This study was designed to answer the following scientific questions using a pig model: (1) Does the SRBAL improve survival in posthepatectomy ALF? (2) Does the SRBAL clear

ammonia, or other toxic waste molecules, in the setting of posthepatectomy ALF? and (3) Does the SRBAL promote liver regeneration?

Materials and Methods

ANIMALS

Resection animals used in this study were wild-type female large white swine weighing 26 kg–30 kg (mean 28.0 ± 1.2 kg). Hepatocyte donors were wild-type female large white swine weighing 12 kg–20 kg (mean 16.7 ± 2.9 kg). All animals were obtained from a local vendor (Manthei Hog Farm, Elk River, MN). All animal husbandry and procedures were performed in accordance with the guidelines set forth by the Mayo Institutional Animal Care and Use Committee.

EXPERIMENTAL TIMELINE

The experimental timeline is outlined in Fig. 1. All animals were fasted at least 8 hours before any surgical procedure. All animals first underwent ambulatory intracranial pressure (ICP) probe placement. One week later, they underwent the following sequence of events: prehepatectomy CT scan, placement of a double-lumen central venous catheter, 85% hepatectomy, and posthepatectomy CT scan. Time 0 ($t = 0$ hours) was defined as the completion of liver resection. After surviving past the initial 24 hours following hepatectomy, 18 animals were randomly assigned to one of the following three treatment groups: standard medical therapy (SMT), no-cell SRBAL (0 g), and 200-g SRBAL (200 g). These animals received continuous treatment for a 24-hour period from $t = 24$ hours to $t = 48$ hours (Fig. 1B). All animals were followed for 90 hours after liver resection or until death equivalence was reached.

STUDY ENDPOINTS

Animals with greater than 500 mL of estimated intraoperative blood loss were excluded from randomization and removed from further study. The study's primary endpoints were survival to 90 hours after resection or deterioration in the first 90 hours to the status of "death equivalence," defined as either grade 4 hepatic encephalopathy (nonresponsiveness to sternal rub, ear tug, or auditory stimulus) while off propofol for at least 1 hour, or inability to maintain sternal posture with an ICP greater than 20 mmHg. Animals that survived 90 hours after hepatectomy were returned to normal housing and monitored for up to 2 weeks (secondary endpoint). Animals not eating, drinking, or ambulating after reaching the 90-hour endpoint were euthanized per Institutional Animal Care and Use Committee policy. Animals that reached a study endpoint were euthanized by sodium pentobarbital overdose.

INTRACRANIAL PRESSURE PROBE PLACEMENT

General anesthesia (induction: 5 mg/kg Telazol, 2 mg/kg Xylazine, and 0.01 mg/kg Glycopyrrolate; maintenance: inhaled isoflurane 1%–2%) was administered to all animals. The ambulatory ICP probe (Raumedic, Germany) was placed following a previously described technique.⁽⁹⁾

DOUBLE-LUMEN CENTRAL VENOUS CATHETER PLACEMENT

A cuffed double-lumen central venous catheter (Palindrome 55 cm, Covidien, Mansfield, MA) was placed in the right internal jugular vein following a previously described technique.⁽⁹⁾

HEPATECTOMY

All animals first underwent general anesthesia. A midline laparotomy from xiphoid to umbilicus was performed with adequate retraction and exposure (Fig. 2A). All peritoneal attachments of the left lateral lobe were released. The portal vein to the left lateral lobe was isolated and ligated with 2–0 silk ties adjacent to the parenchyma (Fig. 2B). The parenchyma was then transected with a cavitron ultrasonic surgical aspirator to expose the major vasculature. The hepatic vein and its branches were carefully isolated and ligated. A DeBakey vascular clamp was applied on the remaining parenchyma, and transection was completed with electrocautery. The remnant was oversewn with 3–0 Prolene (Fig. 2C). Next, the right and left medial lobes were resected together in a similar fashion (Fig. 2D). Finally, peritoneal attachments to the right lateral lobe and posterior inferior vena cava to diaphragm were released (Fig. 2E). Right lateral lobe and caudate lobe were resected one finger breadth lateral to inferior vena cava (Fig. 2F).

ABDOMINAL CT IMAGING AND VOLUMETRICS

All animals underwent abdominal CT imaging immediately before and after hepatectomy. Animals that survived 48 hours, 1 week, and 2 weeks following hepatectomy underwent CT imaging at each of these time points. Intravenous contrast of 60-mL Omnipaque (GE Healthcare, Princeton, NJ) was given through a peripheral ear vein during the first prehepatectomy scan to better define the liver parenchyma. Arterial and portal phases were obtained. No intravenous contrast was administered in subsequent imaging to avoid anaphylactic response from re-exposure. Instead, liver parenchyma was delineated by multiple titanium surgical clips placed intra-operatively at the margins of liver resection.

CT images were uploaded to 3D volumetrics software (TeraRecon, Foster City, CA) and liver volume was calculated following previously described methods.⁽¹⁴⁾ All CT volumetrics results were confirmed by a blinded radiologist.

Volume regeneration in this study was measured by the volume index, defined as volume at any posthepatectomy time point divided by volume immediately following hepatectomy. The volume index at the 48-hour posthepatectomy time point was used to compare regeneration among groups.

STANDARD THERAPY OF POSTHEPATECTOMY PIGS

After the posthepatectomy CT, all animals were taken to the postanesthesia recovery room, where they received continuous IV 5% dextrose normal saline (D5NS) at 80 mL/h. Physical exam and ICP measurement were performed every 2 hours. Propofol up to 1 mg/kg was administered through the central venous line to assure lateral decubitus positioning for all ICP measurements. Reported ICP (mmHg) is the average of 3–5 measurements obtained over a 2-minute interval.⁽⁹⁾ Laboratory values (electrolytes, aspartate aminotransferase

[AST], total bilirubin, creatinine, glucose, international normalized ratio [INR], and ammonia concentration) were obtained every 6 hours. Blood glucose less than 75 mg/dL was treated with 5 mL of 50% dextrose.

All animals were transferred to the operating room suite 24 hours after hepatectomy. Light sedation was provided by continuous propofol drip up to 0.1 mg/kg/min. Heart rate, blood pressure, cutaneous oximetry, and ICP was continuously monitored. All animals received a 1-L warm normal saline bolus and 80 mL/h of D5NS thereafter. This rate was increased as needed to a maximum of 400 mL/h to maintain mean arterial pressure above 50 mmHg. No vasopressor was required. All animals were given an intravenous heparin bolus of 100 units/kg. Blood activated clotting time was measured every 2 hours and heparin was administered intravenously as needed to maintain an activated clotting time level of 175–225 seconds. Laboratory values were obtained every 4 hours.

HEPATOCYTE ISOLATION AND SPHEROID CULTURE

All donor animals were started on 40% protein diet 7 days before harvest.^(19,20) Twenty-four hours prior to planned SRBAL treatment, hepatocytes were isolated from a donor animal using a modified three-step collagenase perfusion method.⁽¹⁹⁾ Viability of isolated hepatocytes was determined by trypan blue exclusion. Spheroids were formed only if viability was greater than 90%. Hepatocyte spheroids were produced by a rocker technique as previously described.⁽¹⁹⁾ Viability of hepatocytes within 3D spheroids was determined by using FluoroQuench staining (One Lambda, Canoga Park, CA).⁽²¹⁾ One to two donor pigs were used to produce a goal of 200 g of hepatocyte spheroids for each treatment.

EXTRACORPOREAL THERAPY

Animals assigned to both extracorporeal therapy groups (0 g and 200 g) were connected to the extra-corporeal device using the two-lumen central venous catheter 24 hours after hepatectomy (Fig. 3). Design of the extracorporeal device and SRBAL was reported in a previous publication.^(9,22) All animals in the 0-g and 200-g groups received continuous treatment for 24 hours.

POSTTREATMENT CARE

After the 48-hour posthepatectomy CT, animals that survived were transferred back to the postanesthesia recovery room. At this point, physical exam and ICP measurement every 2 hours were resumed. Laboratory values were obtained every 6 hours. Animals were provided with 80-mL/h D5NS until they were eating and drinking on their own. Monitoring was continued for up to 90 hours after resection. If the animals were ambulatory, eating, and drinking at 90 hours following hepatectomy, they were returned to their usual housing, and were evaluated daily up to 2 weeks after resection.

TISSUE EXAMINATION

Liver tissues were obtained during hepatectomy and immediately after euthanization. These tissues were formalin-fixed and paraffin-embedded for permanent staining by hematoxylin and eosin (H&E) and immunohistochemical staining with an anti-Ki67 antibody following a previously described protocol.⁽⁹⁾ Images were analyzed at 4 times, 10–20 times, and 40-

times power by a liver pathologist in a blinded fashion. Positive nuclear staining for Ki67 was quantified across 10 separate visual fields at 40 times; the mean and SD of these counts were determined.

CYTOKINE

Porcine serum tumor necrosis factor- α (TNF- α) and transforming growth factor- β (TGF- β) cytokine levels were assayed using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN). Interleukin-6 levels were detected using a commercially available porcine cytokine/chemokine magnetic bead panel kit (Millipore, Millford, MA).

STATISTICS

Categorical data are described as counts and percentages; numerical data are described as mean ($\pm$ SD) of the group. Statistical significance among different treatment groups was determined by the chi-square test for categorical data and Student *t* test for quantitative data. If the data were heavily skewed (nonnormal distribution), Wilcoxon rank-sum test was used. CT volumetrics data were analyzed with Student *t* test. The Kaplan-Meier estimator was used for survival after hepatectomy, in which the primary endpoint was described previously. Animals were randomized conditional on surviving 24 hours following hepatectomy, but *t* = 0 was set to be the time of hepatectomy completion. Data were censored at 90 hours following hepatectomy. The log-rank test was used to compare separately the 200 g with SMT, the 200 g with 0 g, and 0 g with SMT groups after determining that the *P* value associated with the overall log-likelihood was less than 0.05. For data recorded longitudinally, the main analysis was of the end-of-study values at 90 hours, and last observation carry forward was used in the case of missing values. A comparison was made at each time point using Student *t* test or Wilcoxon rank-sum test as appropriate. Missing values were anticipated before starting the study in the case of deaths before 90 hours. If baseline measures were available and differed among groups, analysis of covariance (ANCOVA) was performed, adjusting for the baseline value.

Results

ANIMAL CHARACTERISTICS AND OPERATIVE VARIABLES

Animals in the three study groups were similar in weight (*P* = 0.44), resection time (*P* = 0.97), and estimated blood loss (*P* = 0.94). The differences between prehepatectomy hematocrit ($27 \pm 4\%$) and posthepatectomy hematocrit ($26 \pm 7\%$) were not significant, nor was there a significant difference in hematocrit among the three groups (prehepatectomy *P* = 0.36, posthepatectomy *P* = 0.45).

Animals in each group received a similar amount of 0.9% normal saline intraoperatively (mean 1.9 ± 0.5 L, *P* = 0.43).

Prehepatectomy CT Volumetrics—Average prehepatectomy liver volume was 720.2 ± 55.3 mL, and similar among groups (*P* = 0.99, Table 1).

Posthepatectomy CT Volumetrics—Average posthepatectomy volume was 95.7 ± 3.8 mL. Average percent resection was $86.6 \pm 1.2\%$. Average volume difference based on CT volumetrics was 624.5 ± 56.0 mL. Average weight resected was 490.2 ± 52.9 g. The 200-g group had on average slightly larger resections (Table 1), but no significant difference was found among groups in posthepatectomy volume ($P = 0.70$), percent volume resected ($P = 0.87$), volume resected ($P = 0.98$), or weight resected ($P = 0.65$).

SPHEROID INOCULUM

A total of 11 donor animals with an average weight of 16.7 ± 2.9 kg were sacrificed for liver harvest and spheroid formation (Supporting Table S1). Average harvest yielded 219.3 ± 71.0 g of primary hepatocytes per pig, with an average hepatocyte viability of $96.2 \pm 2.4\%$. The inoculated mass of spheroids in the 6 treated animals averaged 207.8 ± 21.8 g.

SURVIVAL

Survival rate at 90 hours was significantly higher in the 200-g group (5 of 6, 83%) compared with the SMT group (0 of 6, 0%, $P = 0.006$) or the 0-g group (0 of 6, 0%, $P = 0.009$) (Fig. 4A). There was no difference in survival between the SMT and 0-g groups ($P = 0.88$).

All animals in both control groups reached a death-equivalent endpoint between 38 and 51 hours following hepatectomy. These animals universally presented with decreased propofol requirement and violent limb paddling approximately 4–6 hours prior to time of death. These findings were followed by decerebrate posturing with stiffening and extension of neck and limbs. As ICP rose above 20 mmHg, untreated ALF pigs became unresponsive to a painful stimulus and their pupils became fixed and dilated. Pigs were euthanized at this point to avoid respiratory arrest or cardiovascular death. In contrast, 5 of 6 animals treated with the 200-g SRBAL survived to 90 hours following hepatectomy without a significant rise in blood ammonia or signs of cerebral edema. One animal (SRBAL 5) in the 200-g SRBAL treatment group experienced rising blood ammonia levels, elevated ICP, and signs of brain herniation at 44 hours following hepatectomy. All 5 animals surviving to 90 hours were ambulating at that time point. Two of the 5 survivors were euthanized at 90 hours following hepatectomy, as they had not eaten or drank fluids for the previous 24 hours. These 2 animals showed signs of fatigue, ileus, peripheral edema, and ascites consistent with portal hypertension from small-for-size liver graft. The other 3 survivors were followed to 14 days following hepatectomy; they ate, drank, and behaved normally at the time of euthanization on day 14.

LABORATORY VALUES

Ammonia and ICP—Plasma ammonia levels are stratified by group (Fig. 4B). There was no significant difference among groups in baseline (21 ± 13 μ M, $P = 0.34$), immediate posthepatectomy (155 ± 53 μ M, $P = 0.66$) plasma ammonia levels. Pretreatment values obtained at 24 hours following hepatectomy showed that the 200-g group had significantly lower ammonia ($P = 0.003$). ANCOVA was performed, and after adjusting for the 24-hour ammonia value, the 200-g group had significantly lower levels of ammonia from hours 32 to 48 ($P = 0.01$) compared with the SMT and from hours 40 to 48 ($P = 0.01$) compared with the

0-g groups. There was no significant difference between the SMT and 0-g groups at any time point.

ICP values are stratified by group (Fig. 4C). There was no significant difference among groups at baseline and at immediate posthepatectomy (4 ± 3 mmHg, $P = 0.7$) and pretreatment (7 ± 3 mmHg, $P = 0.5$) periods. The 200-g group had significantly lower ICP compared with the SMT group at 48 (10 ± 8 mmHg versus 26 ± 7 mmHg, $P = 0.002$) hours following hepatectomy and with the 0-g group at 44 (9 ± 8 mmHg versus 18 ± 4 mmHg, $P = 0.04$) and 48 (10 ± 8 mmHg versus 21 ± 1 mmHg, $P = 0.007$) hours following hepatectomy. There was no significant difference between the SMT and 0-g groups at any time point.

INR and Heparin Requirement—INR values are stratified by group (Fig. 4D). There was no significant difference among the baseline (1.1 ± 0.2 , $P = 0.3$), immediate posthepatectomy (1.1 ± 0.2 , $P = 0.3$), and pretreatment (2.4 ± 0.3 , $P = 0.7$) values. There were significant differences between the 200-g and 0-g groups at 36 (3.1 versus 2.8, $P = 0.03$) and 48 (3.4 versus 2.6, $P = 0.01$) hours following hepatectomy, but these differences were not seen between the 200-g and SMT groups.

The 200-g group had higher average heparin requirements compared with the 0-g group, but this difference did not reach statistical significance ($24,158 \pm 4,697$ units versus $19,283 \pm 7,407$ units, $P = 0.2$). The SMT group ($13,925 \pm 3,940$ u) required significantly less heparin than the 200-g group ($P = 0.007$); these differences were likely due to metabolism of heparin by hepatocytes in the SRBAL.

AST

Serum AST values are stratified by group (Fig. 4E). AST levels in all groups were comparable at baseline (26 ± 8 U/L, $P = 0.8$) and in the immediate posthepatectomy (153 ± 63 U/L, $P = 0.7$) and pretreatment (666 ± 167 U/L, $P = 0.08$) periods. The 0-g BAL group had the highest average AST values, but no significant difference was identified at any time point.

Total Bilirubin—Serum total bilirubin values are stratified by group (Fig. 4F). Bilirubin levels were similar in all groups at baseline (0.2 ± 0 mg/dL) and in the immediate posthepatectomy (0.2 ± 0.1 mg/dL, $P = 0.4$) and pretreatment (2.0 ± 0.7 mg/dL, $P = 0.7$) periods. No significant difference was identified at any time point.

Laboratory Values in Survivors After 48 Hours Following Hepatectomy—

Laboratory values, including plasma ammonia, ICP, INR, AST, and total bilirubin, of the 5 survivors from 48 to 90 hours following hepatectomy (Supporting Figure S1) trended toward normal limits after 200-g SRBAL treatment.

Cytokines—No significant differences in serum cytokine levels were identified among groups at any point. TNF- α showed a general downward trend following hepatectomy, with the 200-g group having the lowest average level. After 90 hours, the TNF- α level began to trend toward baseline in the 200-g group. Interleukin-6 increased following hepatectomy, with the 200-g group reaching the highest average level among the three groups and trended

down to baseline after 90 hours. TGF- β was increased at 24 hours following hepatectomy and began to trend downward as well. The lowest average levels were found in the 200-g group and began to trend toward baseline after 90 hours. Data are provided in Supporting Table S2.

CT VOLUMETRICS

Posttreatment (48 Hour)—After treatment, 48-hour posthepatectomy volume was stratified by groups (Table 2). The 200-g SRBAL group showed significantly increased 48-hour posthepatectomy volume compared with both control groups (versus SMT, $P = 0.02$; versus 0 g BAL, $P = 0.04$). No significant difference was found between the two control groups ($P = 0.94$).

Volume Regeneration—Volume index at 48 hours, defined as volume at 48 hours divided by volume immediately following hepatectomy, was used to compare regeneration among groups. The 200-g group showed a volume index of 2.21, which was significantly higher than the SMT ($P = 0.01$) and 0-g ($P = 0.03$) groups (Table 2). No significant difference was found between the 0-g and SMT groups ($P = 0.88$).

HISTOLOGY

Ki-67—At baseline, $1.9 \pm 1.0\%$ of hepatocytes in a 40-times visual field were stained positive for Ki-67 (Fig. 5A). In the SMT group, in which tissue samples were obtained 38–51 hours after hepatectomy, $44.0 \pm 14.7\%$ ($P < 0.01$ versus baseline) of hepatocytes were Ki-67 positive (Fig. 5B). In the 0-g group, in which tissue samples were obtained 41–48 hours after hepatectomy, $43.7 \pm 18.6\%$ ($P < 0.01$ versus baseline) of hepatocytes were Ki-67 positive. In the 200-g group, the only nonsurvivor had 50% positive Ki-67 staining at 44 hours following hepatectomy, but the remaining 5 survivors had $9.0 \pm 6.3\%$ ($P = 0.08$ vs. baseline) positive Ki-67 staining beyond 90 hours after hepatectomy (Fig. 5C).

H&E—All histology samples were examined by a blinded pathologist. No evidence of liver pathology was observed at baseline. No significant liver injury or necrosis was observed in any of the post euthanasia samples. However, marked sinusoidal dilatation was observed in all posthepatectomy samples, consistent with the clinical finding of portal hypertension (Fig. 5D,E).

SPHEROID PERFORMANCE

Ammonia Clearance—In the 0-g and 200-g groups, reservoir ammonia concentration was measured every 4 hours starting at 28 hours after hepatectomy. Significant differences in ammonia concentration between the two groups were seen at 40 hours following hepatectomy and beyond (Supporting Fig. S2A). Ammonia clearance was defined as micromoles of ammonia cleared by the reservoir per minute (Supporting Fig. S2B).

O₂ Consumption—Oxygen consumption was used as an indicator of spheroid health (Supporting Fig. S2C). Oxygen consumption trended down gradually throughout the treatment period from $133.7 \pm 46.6 \mu\text{M}/\text{min}$ at 28 hours following hepatectomy to $102.7 \pm 60.2 \mu\text{M}/\text{min}$ at 48 hours following hepatectomy.

Discussion

This study evaluates a BAL in the setting of posthepatectomy ALF in pigs. Our porcine hepatectomy model was developed based on modifications of previously described techniques.^(23,24) Liver resection was completely guided by anatomical landmarks to avoid potential surgeon bias. As a result, the model was very reproducible. This has not been true of past large animal models of ALF.^(22–25) All nonsurvivors followed a particular and predictable clinical course, and reached death equivalent between 38 and 51 hours following hepatectomy.

This study was designed to answer three scientific questions. The first question addressed the potential survival benefit of SRBAL treatment. The SRBAL with 200 g of hepatocyte spheroids was associated with significant survival benefit in posthepatectomy ALF. The 200-g group reached an 83% 90-hour survival rate compared with 0% in both the SMT and 0-g groups. There was no difference in survival or clinical progression observed between the 0-g and SMT groups. One animal in the 200-g SRBAL group (SRBAL5) experienced brain herniation at 44 hours following hepatectomy. This animal had no deviation from a volume-resected, posthepatectomy care, or pretreatment laboratory evaluation point of view. However, the harvest of hepatocytes used to treat this animal was suboptimal, with low yield (121.0 g) and low viability (93%). We hypothesize that cell quality was reduced by the donor animal's large size (Supplementary Table 1, SRBAL5 Donor A) and reduced organ perfusion, as our procurement protocol had been optimized for smaller animals. One challenge to overcome in this study was the variability of harvest yield (121.0 g–327.1 g) and viability (91%–98%). The technical aspect of the procedure was well established and primary hepatocyte to spheroid conversion had been consistently 60%–70%. We attribute this variability to lot-to-lot variability of the collagenase activity, which was difficult to control. Moving forward, we plan to use rigorous criteria for selection of donor animals, harvest yield, and harvest viability to ensure excellent quality of hepatocyte spheroids used in future clinical trials of SRBAL.

Five of the 6 pigs treated with the 200-g SRBAL survived to the 90-hour primary endpoint. All 5 of these ALF pigs were neurologically normal at 90 hours but had developed signs of small-for-size syndrome including ascites, peripheral edema, fatigue, and anorexia. Two of the 5 survival pigs had developed severe ascites and were unable to meet IACUC requirements for oral intake beyond 90 hours following hepatectomy and were therefore euthanized. The other 3 survivors were able to tolerate oral intake in the setting of less severe small-for-size symptoms, which gradually subsided over time. These 3 animals had returned to normal appetite and activity when they were euthanized at 2 weeks following hepatectomy.

The second question was whether SRBAL treatment provided satisfactory detoxification of ammonia to prevent cerebral edema in the setting of posthepatectomy ALF. Cerebral edema is a characteristic and feared complication of ALF that can result in uncal herniation and brain death.⁽²⁶⁾ Cerebral edema has been strongly associated with blood ammonia levels,⁽²⁷⁾ and survival benefits with reduction in ammonia levels in large animal ALF models have been demonstrated previously.^(6–9) Our data support these prior results, namely, that 200-g

SRBAL treatment reduced blood levels of ammonia significantly throughout the entire 24-hour treatment interval compared with the 0-g and SMT groups. Reduced ammonia was strongly associated with avoidance of cerebral edema, lower ICP levels, and improved survival. Brain water was not measured in this study but we have previously reported a correlation between brain water content and ICP level, used as an indirect measure of cerebral edema and neuroprotection during SRBAL treatment.^(9,28)

Ammonia level at 24 hours following hepatectomy showed significantly lowered pretreatment ammonia levels in the 200-g group, which was unexpected. All animals underwent a strictly standardized resection procedure and postoperative management. Liver volume resected was verified by CT volumetrics to be comparable among groups. We believe this observation is a result of random occurrence in a study with a small study population. Nevertheless, after adjusting for the 24-hour value with ANCOVA analysis, the 200-g group maintained significantly lowered ammonia level compared with both control groups. The ideal experiment setup to avoid potential bias would involve two blinded teams, in which one performs the hepatectomy and the other manages treatment afterward. However, in reality, this would require massive manpower and time to achieve.

Our 0-g SRBAL group was designed to assess the effect of an extracorporeal albumin dialysis circuit in the setting of posthepatectomy ALF, in the absence of primary hepatocyte spheroids. Albumin dialysis was previously shown to decrease ICP and white-matter water content without reduction of serum ammonia level.⁽²⁹⁾ However, we did not observe a benefit of a purely albumin-based circuit in our study, as the 0-g SRBAL treatment showed no change in survival or intracranial pressure from the SMT group. In fact, blood ammonia levels were higher in the 0-g SRBAL group compared with the SMT group, possibly related to ammonia production from the albumin circuit.

The third question this study aimed to answer is whether SRBAL treatment promoted liver regeneration. CT volumetrics data demonstrated a significantly larger liver volume and higher volume index at 48 hours following hepatectomy in the 200-g SRBAL group compared with the two control groups. This observation is more impressive considering that the 200-g SRBAL group underwent a larger resection with lower average posthepatectomy volume (not statistically significant) compared with the SMT and 0-g groups (Table 1). One limitation of this study is the inability to sample liver from treated animals at a time point corresponding to death of controls. Therefore, Ki-67 staining in this study was able to show a marked increase of regeneration around 48 hours following hepatectomy but was not useful in determining SRBAL treatment effects. The best way to address this problem is to design a separate study with a similar setup, but in which all animals would be euthanized at 48 hours following hepatectomy so the tissues can be obtained and compared for treatment effects.

Histology of the posthepatectomy liver remnant showed minimal necrosis and rare hepatocyte apoptosis. These findings were highly reproducible and consistent with the lack of TNF- α increase. Our observations are in contrast with previously reported animal models of toxin-induced liver failure.^(9,30) Serum cytokines levels of ALF pigs in the treatment group and control groups of the current study showed no differences (Supporting Table S2),

suggesting that the benefit of SRBAL therapy was not anti-inflammatory, but rather a neuroprotective effect of improved ammonia detoxification by the hepatocyte-based extracorporeal circuit. If the SRBAL provided an anti-inflammatory benefit, it was most likely not detected due to the small sample size.

SRBAL demonstrated powerful performance in survival improvement and ammonia detoxification in medical and surgical ALF pigs with potential benefits in stimulating hepatocyte regeneration. The possibility of SRBAL as a preemptive therapy on patients who undergo extensive liver resections or small-for-size liver transplantation was not addressed but is another potential treatment population. SRBAL is moving toward clinical trials and the results are highly anticipated. However, currently, no device has been shown to have a significant survival benefit in human clinical trials, albeit promising results in preclinical settings. In all preclinical trials, the ALF models were designed to have a single etiology (toxin-induced or posthepatectomy) that led to a single cause of death: cerebral edema. Extracorporeal therapy devices were designed particularly to detoxify ammonia and prevent mortality from cerebral edema. In reality, the ALF patient population has extremely heterogeneous etiologies and clinical presentations. In addition to cerebral edema, ALF patients suffered morbidity and mortality from various causes, such as bleeding and infection. Therefore, to prove survival benefits of SRBAL in ALF patients with risks of complications from cerebral edema, a rigorously designed randomized control trial with clearly defined inclusion criteria, plus the support of an excellent intensive care team, is required.

In summary, the SRBAL proved to be beneficial in treating pigs experiencing posthepatectomy ALF. SRBAL treatment resulted in high levels of ammonia detoxification and neuro-protection in posthepatectomy ALF pigs, similar to our previous report in the setting of drug-induced porcine ALF.⁽⁹⁾ Furthermore, increased liver regeneration associated with the 200-g SRBAL treatment was confirmed by CT volumetrics imaging. Further studies are needed to delineate the specific mechanism(s) of enhanced regeneration. Randomized controlled clinical trials are warranted to determine the benefits of SRBAL treatment in clinical populations of ALF patients, including those who are at risk for ALF following hepatectomy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ALF acute liver failure

ANCOVA	analysis of covariance
AST	aspartate aminotransferase
BAL	bio-artificial liver
CT	computed tomography
D5NS	5% dextrose in normal saline
H&E	hematoxylin and eosin
ICP	intracranial pressure
INR	international normalized ratio
SMT	standard medical therapy
SRBAL	spheroid reservoir bio-artificial liver
TGF-β	transforming growth factor β
TNF-α	tumor necrosis factor α

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FIG. 1.

Experiment timeline. (A) Each animal was studied for 2 weeks. Each animal underwent ambulatory ICP probe placement during the first week, and underwent 85% hepatectomy, randomized treatment, and 90-hour observation in the second week. (B) Timeline of 90-hour observation in second week.

FIG. 2.

Intra-operative photos depicting steps of 85% hepatectomy. (A) Porcine liver in natural position. (B) Left lateral lobe vasculature (blue: portal vein; white: hepatic vein) isolation. (C) Left lateral lobe removed and remnant oversewn with 3-0 Prolene. (D) Resection of left medial and right medial lobes. (E) Right lateral lobe mobilized. (F) Resection of right lateral lobe one finger breadth lateral to inferior vena cava and completion of hepatectomy. Abbreviations: Diaph, diaphragm; HV, hepatic vein; LLL, left lateral lobe; LML, left medial lobe; PV, portal vein; RLL, right lateral lobe; RML, right medial lobe.

FIG. 4.

Kaplan-Meier analysis of survival and comparison of laboratory values among groups. (A) Kaplan-Meier curve of the three groups. Significant survival differences found between 200 g versus 0 g and 200 g versus SMT. (B) Mean plasma ammonia levels in each group. The 200-g group showed significantly decreased ammonia levels compared with the control groups. (C) Mean ICP values in each group. The 200-g group showed significantly decreased ICP level compared with the control groups. (D) Mean INR values in each group. The 200-g group showed significantly decreased INR values compared with the 0-g group.

(E) Mean AST values in each group. No significant differences were identified among groups at any time point. (F) Mean total bilirubin values in each group. No significant differences were identified among groups at any time point. *A significant difference ($P < 0.05$) compared with the 200-g group.

FIG. 5.

Liver histology. (A) Ki-67 staining at baseline. No liver pathology was observed. (B) Nonsurvivor liver Ki-67 staining showed marked increase of positive staining at 48 hours following hepatectomy. (C) Survivor liver obtained at 2 weeks showed positive staining close to baseline. (D) Baseline H&E staining. No Liver pathology was observed. (E) Nonsurvivor liver H&E staining showed sinusoidal dilatation consistent with clinical observation at 48 hours following hepatectomy.

TABLE 1.

Prehepatectomy Volume, Posthepatectomy Volume, Percent Volume Resected, Volume Resected, and Weight Resected Stratified by Group

	SMT	0 g BAL	200 g SRBAL	P value
Prehepatectomy volume (mL)	719 ± 61.1	718.5 ± 71.3	723 ± 39.5	0.99
Posthepatectomy volume (mL)	96.8 ± 4.3	95.4 ± 4.1	94.9 ± 3.6	0.70
Percent volume resected (%)	86.4 ± 1.5	86.6 ± 1.2	86.8 ± 1.1	0.87
Volume resected (mL)	622.2 ± 62.7	623.1 ± 70.0	628.1 ± 42.3	0.98
Weight resected (g)	482.9 ± 41.5	480.4 ± 58.5	507.2 ± 62	0.65

TABLE 2.

Posthepatectomy Volume and Volume Index (48 Hour)

	SMT	0 g BAL	200 g SRBAL
48-hour postoperative volume (mL)	176.2 ± 8.8	175.8 ± 7.8	210.0 ± 25.9
Volume index	1.83 ± 0.07	1.82 ± 0.15	2.21 ± 0.25