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Clinical Experience With a Bioartificial Liver in the Treatment of Severe Liver Failure

A Phase I Clinical Trial

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Objective
The purpose of this study was to develop a bioartificial liver (BAL) to treat patients with severe liver failure until they can be either transplanted or recover spontaneously.

Summary Background Data
Severe acute liver failure is associated with high mortality. Liver transplantation has emerged as an effective therapy for patients who did not respond to standard management. However, because of the donor organ shortage and urgent need for transplantation, many patients die before they can be transplanted and others do not survive after transplantation, primarily because of intracranial hypertension.

Methods
Three groups of patients with severe acute liver failure were treated with the BAL. In group 1 (n = 18) were patients with fulminant hepatic failure (FHF), in group 2 (n = 3) were patients with primary nonfunction (PNF) of a transplanted liver, and in group 3 (n = 10) were patients with acute exacerbation of chronic liver disease. Patients in groups 1 and 2 were candidates for transplantation at the time they entered the study, whereas patients in group 3 were not.

Results
In group 1, 16 patients were "bridged" successfully to transplantation, 1 patient was bridged to recovery without a transplant, and 1 patient died because of concomitant severe pancreatitis. In group 2, all patients were bridged successfully to retransplantation. In group 3, two patients were supported to recovery and successful transplants at later dates; the other eight patients, although supported temporarily with the BAL, later died because they were not candidates for transplantation.

Conclusions
The authors' clinical experience with the BAL has yielded encouraging results. A randomized, controlled, prospective trial (phase II–III) is being initiated to determine the efficacy of the system.

The management of severe acute liver failure remains a major clinical challenge.[1] Although substantial advances have been made in general supportive therapy and critical care, mortality in some forms of acute liver failure, specifically fulminant hepatic failure (FHF), remains unacceptably high.[1] This primarily is because of incomplete understanding of the pathophysiology of the disease in general and FHF in particular. It still is unclear why patients with acute exacerbation of underlying chronic liver disease do not, as a rule, have cerebral edema, whereas patients without chronic liver disease often experience rapid onset of intracranial hypertension.

Despite incomplete understanding of the pathophysiology of the disease, clinicians have attempted to develop rational, novel therapeutic methods.[2] Most attempts focused on plasma detoxification, based on the assumption that "toxins" accumulate in the plasma as a result of failure of the diseased liver to perform key metabolic functions.[3–5] In the past 30 years, however, attempts to provide plasma detoxification by using plasma exchange, specific and nonspecific binding of potentially toxic components in the plasma using various binding techniques (charcoal, affinity columns, resins), have been unsuccessful.[3–7]

Whole liver perfusion has been used successfully to treat patients with FHF and other more chronic forms of liver failure.[8] The major limitation in the case of perfusion with whole human liver remains the shortage of organs; additionally, it can be argued that if a liver is suitable for perfusion, it should be suitable for transplantation. Xenogeneic whole liver perfusion has been shown to be effective.[8,9] The major challenges that need to be resolved with this approach are immunologic and logistic.

Our strategy has been to use intact isolated xenogeneic hepatocytes as a means of achieving both detoxification and provision of absent synthetic liver function. Use of isolated cryopreserved hepatocytes eliminates highly antigenic cell populations present in the whole xenogeneic liver (*i.e.,* endothelial cells and macrophages) and provides a logistically simpler technology that can be made easily accessible. Several investigators have developed and tested a variety of hepatocyte-based systems.[10–15] We have developed a bioartificial liver (BAL) containing porcine hepatocytes and have shown its ability to provide detoxifying and synthetic functions in a series of *in vitro* and *in vivo* animal experiments as well as in pilot clinical studies.[2,16–20] Based on the data collected from these studies, BAL design was optimized and we arrived at the system currently used in the clinical trial described here.

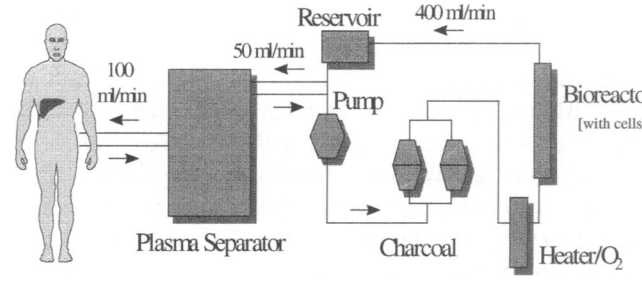

Figure 1. Bioartificial liver circuit connected to plasma separator and patient. Blood is removed from the patient and is separated into plasma and blood cells in a plasma separator. Plasma is perfused through a charcoal column to reduce its toxicity before it perfuses a bioreactor hollow-fiber module containing 5 billion matrix-anchored viable porcine hepatocytes. A temperature regulator and oxygenator are included in the system to provide a physiologic environment for the cells. A volume reservoir allows high recirculation flow through the circuit containing the porcine hepatocytes, thus enhancing the efficiency of the system.

The goal of this study was to establish the safety of the BAL in treating patients with severe acute liver failure, to streamline and standardize the processes of cell harvesting and cryopreservation, to finalize the configuration of the system, and to put in place clinical protocols that would allow meaningful data collection and analysis for assessment of efficacy.

METHODS

Bioartificial Liver Design

Hepatocytes

Methods of porcine hepatocyte isolation, purification, attachment to a matrix, cryopreservation, and storage have been described previously.[2,16–23] Freshly isolated hepatocytes were used to treat the first 15 patients in this series, and cryopreserved cells were used to treat the last 16. No significant differences between the two cell preparations were noted in terms of their physiologic and clinical effects. The mean initial viability of fresh cells was approximately 90%, whereas that of cryopreserved cells was 70%. Five billion viable, fresh or cryopreserved, hepatocytes were used for each patient treatment. All future treatments will use cryopreserved cells.

System Characteristics

The design of the current BAL system (Fig. 1) has evolved from a series of *in vitro* and *in vivo* experimental animal and pilot clinical studies carried out over a period

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of several years.[2,16–23] The system has been standardized and in the last 24 patients of this series, we used a BAL circuit (HepatAssist 2000; Circe Biomedical, Lexington, MA). Blood is removed from a patient through a double-lumen catheter placed in the superficial femoral vein. Blood then is separated into blood cells and plasma using a Spectra plasma separator (Cobe, Lakewood, CO). Plasma enters the BAL system, which consists of a high-flow plasma recirculation loop through a reservoir, a column loaded with activated cellulose-coated charcoal (Adsorba 300C; Gambro, Hechingen, Germany), an oxygenator (Capiox 308; Terumo Inc., Tokyo, Japan), a heater (Temp Marq, Marquest, Lakewood, CO), and a hollow-fiber module containing hepatocytes. The hollow-fiber module (Microgon, Laguna Hills, CA) is made of porous (0.2 μm) cellulose acetate fibers with a total internal surface area of 6000 cm^2, external surface area of 7000 cm^2, wall thickness of 62 μm, and extra-fiber volume of 200 mL. Five billion viable porcine hepatocytes attached to collagen-coated dextran microcarriers (Cytodex 3; Pharmacia, Piscataway, NJ) are seeded into the extra-fiber space through a side port. Plasma circulates through the fibers at 400 mL/minute. There is free exchange of macromolecules across the surface of the fibers. The primary mechanism of solute transfer is fluid convection by a transfiber pressure gradient. As a safety measure, pressure in the system is monitored to alert the operator if an unexpected leak or blockage were to occur. Sodium citrate is used as an anticoagulant to prevent thrombosis within the circuit. Recently, we have introduced a more efficient hollow-fiber module consisting of polysulfone fibers (Circe Biomedical, Lexington, MA). After return from the BAL, plasma and blood cells are reconstituted and returned to the patient by way of the double-lumen venous catheter.

Clinical Study Design

Patient Groups

All procedures were conducted in full compliance with the standards of the Institutional Committee for the Protection of Human Subjects, in accordance with the Helsinki Declaration of 1975. Patients belonged to one of three groups. Group 1 (n = 18) patients had no history of chronic liver disease, fulfilled all diagnostic criteria of FHF, and were candidates for orthotopic liver transplantation (OLT) at the time of admission to our unit. Group 2 (n = 3) patients had undergone OLT and had primary nonfunction (PNF) of the transplanted liver develop in the immediate postoperative period with rapid clinical deterioration. Group 3 (n = 10) patients presented with acute exacerbation of an underlying chronic liver disease process and were not candidates for OLT at the time of admission. Patients in groups 1 and 2 were evaluated immediately for OLT and were placed on a national waiting list for urgent (United Network of Organ Sharing status 1) transplantation. Patients in group 3 were not candidates for OLT at the time they entered the study, mostly because of active alcohol use, sepsis, and multiorgan failure.

Patient Demographics

The demographic characteristics of the patients are summarized in Table 1. Patients entered the study when stage 3 encephalopathy developed[24] while they were receiving standard medical therapy. With the exception of one patient who was stage 3 encephalopathic, all patients in group 1 were in stage 4 encephalopathy (deep coma) and exhibited signs of brainstem dysfunction and decerebration (*i.e.,* posturing, disconjugate eye movements, anisocoria, sluggish pupil reactivity) because of intracranial hypertension. All three patients in group 2 were stage 4 encephalopathic. Patients in group 3 were stage 4 encephalopathic with the exception of two patients who were in stage 3. Patients were excluded from the study if they were unstable hemodynamically and had overt signs of sepsis. Patients were enrolled in group 1 only if they were candidates for OLT and in group 2 if they were candidates for retransplantation.

Table 2 summarizes the etiology of liver failure. The term "indeterminate" refers to patients who clinically appear similar to the ones with FHF of viral etiology, but no viral hepatitis or other markers can be detected. Ischemic etiology refers to patients with low flow to the liver secondary to another process, that is, dehydration due to heatstroke and pancreatitis.

Liver Support Unit

We have established a multidisciplinary Liver Support Unit to provide optimal, focused care to patients with severe acute liver failure. The clinical team consists of physicians, nurses, pharmacists, nutritionists, physical and respiratory therapists; various areas are represented (hepatology, transplantation, anesthesiology, infectious diseases, nephrology, neurology, neurosurgery, critical care, pulmonology). The research team consists of cell and molecular biologists, neurophysiologists, hepatic physiologists, experimental surgeons, and engineers. The interaction between the research and clinical teams allows seamless transfer of technology from the laboratory to the bedside. Patients are admitted to a dedicated Surgical intensive care unit area. In addition to using a computerized online clinical data collection system (CareVue 9000 Clinical Information System; Hewlett–Packard, Andover, MA), we have developed and implemented standardized diagnostic and therapeutic clinical protocols to allow meaningful and accurate data collection and analysis.

Table 1. PATIENT DEMOGRAPHIC CHARACTERISTICS

Group	Mean Age (yr)	Sex (male/female)	No. of BAL Treatments	"Bridge" Time (hr)
I	36.1 ± 3.4	7/11	2.1 ± 0.2	45.3 ± 5.7
II	48.3 ± 11.2	1/2	1.6 ± 0.6	83.0 ± 54.6
III	51.4 ± 3.5	7/3	1.8 ± 0.3	89.0 ± 31.0

BAL = bioartificial liver.

Treatment Parameters–Conditions

Immediately on admission to the Liver Support Unit, patients undergo rapid clinical evaluation. Invasive hemodynamic monitoring (peripheral arterial catheter and pulmonary artery catheter) is instituted after rapid blood product infusion to correct partially the coagulopathy. A nasogastric tube and a bladder catheter are placed. Patients with signs of intracranial hypertension have an intracranial pressure (ICP) monitor placed at the bedside (subdural) for ICP and cerebral perfusion pressure (CPP) monitoring. Patients are intubated endotracheally when in stage 4 encephalopathy and are placed on ventilator support. The head of the bed is elevated, and the room is kept dark and quiet in the case of patients with intracranial hypertension. Gastric pH is monitored, and patients begin receiving ulcer prophylaxis. All standard supportive medical therapy is instituted (*i.e.,* hyperventilation, mannitol, lactulose). Supportive measures are initiated to correct electrolyte (*i.e.,* hypocalcemia), metabolic (*i.e.,* hypoglycemia, lactic acidemia), respiratory, coagulation, and hemodynamic abnormalities. Renal dialysis and filtration are used as needed. In patients with neurologic dysfunction, a head computed tomographic scan is obtained whenever possible.

Vital signs, urinary output, and central hemodynamic parameters are monitored continuously. Neurologic monitoring and assessment consisting of hourly clinical examination, serially assessing brainstem dysfunction by using the comprehensive level of consciousness score (CLOCS) system, determination of the Glasgow coma score, and continuous monitoring of ICP and CPP, are carried out in all patients with clinical evidence of brainstem dysfunction. Liver function tests, coagulation tests, complete blood cell count, serum electrolytes, ammonia, lactate, blood urea nitrogen, and creatinine levels are determined at the start, midpoint, and end of each BAL treatment. Arterial blood gases are determined serially.

A double-lumen catheter is placed in the right superficial femoral vein for BAL treatments (and renal dialysis if needed). Each BAL treatment lasts 6 hours and is preceded by 1 hour of plasma separation (not exchange) to ascertain patient stability and ensure that the patient tolerates the extracorporeal circuit. Sodium citrate is used as an anticoagulant to prevent thrombosis within the BAL circuit. Plasma-ionized calcium levels are measured hourly during BAL treatment to prevent ionized hypocalcemia due to the calcium-chelating effect of citrate. Calcium chloride infusion is initiated at the start of BAL treatment. Blood is removed from the patient at approximately 90 to 100 mL/minute, and it is separated into blood cells and plasma. The plasma enters the BAL circuit at 50 mL/minute and then the plasma reservoir. Plasma recirculates through the BAL at 400 mL/minute. Plasma is maintained at physiologic temperature and is oxygenated. Finally, plasma is reconstituted with blood cells and returns to the patient at the same rate at which it is removed (90–100 mL/minute). The complete system is shown diagrammatically in Figure 1.

Data Analysis

Statistical analysis was carried out using the paired Student's t test to compare immediately pre- and post-

Table 2. DISEASE ETIOLOGY

Group	Viral	Indeterminate	Acetaminophen	Alcoholic	Ischemic	Autoimmune	PBC
I	4	8	4		2		
II	1	1				1	
III	3	1		4		1	1

PBC = Primary biliary cirrhosis.

Table 3. EFFECT OF BAL TREATMENT ON HEMODYNAMIC PARAMETERS

	Pre-BAL	Post-BAL	p
Group I			
Heart rate (beats/min)	99 ± 3	99 ± 2	<0.8
MAP (mmHg)	86 ± 2	83 ± 2	<0.1
CVP (cmH_2O)	8.7 ± 0.8	9.0 ± 0.7	<0.6
Cardiac index	5.0 ± 0.3	4.4 ± 0.2	<0.1
Group II			
Heart rate (beats/min)	102 ± 5	91 ± 3	<0.1
MAP (mmHg)	79 ± 3	88 ± 6	<0.2
CVP (cmH_2O)	14.0 ± 2.0	14.9 ± 3.0	<0.9
Cardiac index	6.5 ± 2.5	6.4 ± 2.6	<0.7
Group III			
Heart rate (beats/min)	100 ± 3	100 ± 3	<1.0
MAP (mmHg)	81 ± 3	82 ± 4	<0.7
CVP (cmH_2O)	12.4 ± 1.4	14.0 ± 1.3	<0.2
Cardiac index	5.8 ± 0.4	5.4 ± 0.4	<0.1

BAL = bioartificial liver; MAP = mean arterial pressure; CVP = central venous pressure.

BAL treatment values. A p value < 0.05 is considered significant. Data are expressed as means plus standard errors.

RESULTS

Bioartificial Liver Treatment

Patients tolerated BAL treatments well. With the exception of one patient who experienced an episode of transient hypotension requiring discontinuation of a treatment, patients remained stable hemodynamically during treatments (Table 3). No technical problems were identified during plasma separation and BAL perfusion (*i.e.*, embolization, thrombosis, bleeding). All patients were supplemented with calcium chloride to prevent ionized hypocalcemia. There were no adverse hypersensitivity or immunologic reactions to the use of porcine hepatocytes. There was no evidence that treatment with the BAL had an adverse effect on subsequent liver allograft survival and function.

Patient Survival

Group 1 (n = 18)

All patients who were candidates for OLT were "bridged" successfully to OLT (n = 17). One patient with FHF due to ischemic liver injury after a heat stroke initially was a candidate for transplantation, underwent five treatments with the BAL, and showed remarkable neurologic recovery; however, severe ischemic necrotizing pancreatitis subsequently developed, and he was removed from the transplant list and died 21 days later because of multiorgan failure. Sixteen of the 17 patients who were bridged successfully to OLT have experienced full neurologic recovery and were discharged from the hospital. One patient treated with the BAL recently underwent OLT and currently is in the intensive care unit. One patient was treated with the BAL and recovered fully without undergoing OLT.

Group 2 (n = 3)

All three patients in this group were bridged successfully to retransplantation, experienced full recovery, and have been discharged from the hospital.

Group 3 (n = 10)

Patients in this group experienced transient clinical improvement after BAL treatment. However, only two patients were able to recover enough native liver function to survive the acute-on-chronic failure; these two patients later became candidates for OLT (in one case after treatment of an infection, and in the other, after entering an alcohol rehabilitation program for 6 months). The remaining eight patients died 1 to 21 days (mean, 7.1 days) after their last BAL treatment from variceal bleeding and multiorgan failure.

Neurologic Effects of the BAL

Patients in group 1 experienced remarkable neurologic improvement with reversal of the decerebrate state after BAL treatments; posturing, anisocoria, sluggish pupil re-

Table 4. EFFECT OF BAL ON NEUROLOGIC PARAMETERS

	Pre-BAL	Post-BAL	p
Group I			
ICP (mmHg)	17.0 ± 1.7	10.0 ± 1.2	<0.0002
CPP (mmHg)	67 ± 3	73 ± 2	<0.05
GCS	6.6 ± 0.4	7.2 ± 0.4	<0.05
CLOCS	24.7 ± 1.3	31.0 ± 1.1	<0.00005
Group II			
GCS	5.0 ± 1.1	7.0 ± 1.4	<0.2
CLOCS	29.7 ± 7.4	31.7 ± 7.9	<0.5
Group III			
ICP (mmHg)	12.3 ± 0.9	14.0 ± 1.5	<0.4
CPP (mmHg)	85 ± 1	98 ± 8	<0.3
GCS	8.2 ± 0.7	8.4 ± 0.7	<0.4
CLOCS	29.7 ± 2.3	34.0 + 1.7	<0.001

BAL = bioartificial liver; ICP = intracranial pressure; CPP = cerebral perfusion pressure; GCS = Glasgow Coma Score; CLOCS = comprehensive level of consciousness score.

Table 5. EFFECT OF BAL ON LIVER FUNCTION TESTS

	Pre-BAL	Post-BAL	p
Group I			
AST (U/L)	1036 ± 283	695 ± 153	<0.02
ALT (U/L)	955 ± 216	596 ± 144	<0.0007
Alkaline phosphatase (U/L)	118 ± 9	90 ± 8	<0.0006
Total bilirubin (mg/dL)	21.5 ± 1.8	17.0 ± 1.5	<0.0001
Direct bilirubin (mg/dL)	10.4 ± 1.2	7.7 ± 0.8	<0.0002
Indirect bilirubin (mg/dL)	11.1 ± 1.0	9.3 ± 0.9	<0.0001
Group II			
AST (U/L)	5661 ± 2613	2821 ± 1291	<0.1
ALT (U/L)	2139 ± 704	1633 ± 544	<0.05
Alkaline phosphatase (U/L)	108 ± 15	83 ± 9	<0.03
Total bilirubin (mg/dL)	19.1 ± 2.2	14.7 ± 1.7	<0.009
Direct bilirubin (mg/dL)	3.8 ± 1.3	3.1 ± 0.8	<0.2
Indirect bilirubin (mg/dL)	15.3 ± 2.3	11.6 ± 1.3	<0.05
Group III			
AST (U/L)	692 ± 374	723 ± 409	<0.5
ALT (U/L)	349 ± 126	281 ± 114	<0.06
Alkaline phosphatase (U/L)	117 ± 10	119 ± 22	<0.9
Total bilirubin (mg/dL)	26.0 ± 2.7	21.6 ± 2.2	<0.00003
Direct bilirubin (mg/dL)	12.8 ± 1.5	10.3 ± 1.2	<0.00006
Indirect bilirubin (mg/dL)	13.2 + 1.8	11.3 ± 1.5	<0.002

BAL = bioartificial liver; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

activity were lessened, and patients became more responsive to external stimuli. Brainstem function improved, as shown by the higher CLOCS scores (Table 4). There was a significant reduction in ICP with a concomitant increase in CPP (Table 4). This can be better appreciated if one examines more closely patients whose ICP was high. In 16 BAL treatments in which the ICP was >20 mmHg, the effect of BAL treatment was dramatic (ICP: 21.6 ± 2.1 to 8.7 ± 2.6 mmHg, $p < 0.004$; CPP: 67.7 ± 4.2 to 79.7 ± 4.1 mmHg, $p < 0.008$; CLOCS: 19.2 ± 1.2 to 27.1 ± 1.4, $p < 0.0001$).

It is difficult to quantitate the neurologic impact of BAL treatment in patients in group 2 because they were in the postanesthetic period under the influence of paralyzing drugs and other medications. Patients in group 3 experienced transient neurologic improvement after BAL treatments, manifested primarily by increased responsiveness. This observation, however, was difficult to quantitate. There was an improvement in the CLOCS score due to the effect seen in a small number of patients in this group who experienced a degree of brainstem dysfunction.

Other Effects of the Bioartificial Liver

The effects of BAL treatment on liver function tests, metabolic, renal function, hematologic, and coagulation parameters are listed in Tables 5 through 7. A decrease in ammonia, transaminases, and bilirubin levels was observed. No improvement in prothrombin time or other coagulation parameters was seen, but most of the patients already had been treated with fresh frozen plasma and clotting factor concentrates. Plasma amino acid levels were measured at the beginning and end of each BAL treatment. The ratio of branched chain amino acids (*e.g.*, leucine, isoleucine, valine) to aromatic amino acids (*e.g.*, phenylalanine, tyrosine) thought to be an index of the degree of encephalopathy, was calculated.[25] There was a significant ($p < 0.01$) increase (improvement) in the branched chain amino acid–aromatic amino acid ratio from 0.75 ± 0.07 to 0.98 ± 0.07. The increase primarily was because of a reduction in aromatic amino acid levels.

DISCUSSION

Multiple attempts have been made to develop extracorporeal and other systems to provide support for the failing liver.[3–21] The strategies and the pros and cons of the various systems have been discussed previously.[2,22] The complexity of the liver is such that we are unable to provide full replacement of all liver functions for any significant length of time with any of the existing systems, including *ex vivo* perfusion with intact human liver. The question then becomes: what are the relatively few critical liver functions that need to be supported to improve patient survival?

Table 6. EFFECT OF BAL ON METABOLIC AND RENAL FUNCTION PARAMETERS

	Pre-BAL	Post-BAL	p
Group I			
Glucose (mg/dL)	128 ± 7	171 ± 10	<0.00006
Ammonia (μmol/L)	155 ± 10	130 ± 8	<0.007
Lactate (mmol/L)	4.8 ± 1.0	4.5 ± 1.0	<0.2
Albumin (g/dL)	3.2 ± 0.1	2.6 ± 0.1	<0.000001
BUN (mg/dL)	21.0 ± 3.4	18.8 ± 3.0	<0.0003
Creatinine (mg/dL)	1.8 ± 0.2	1.4 ± 0.2	<0.0001
Group II			
Glucose (mg/dL)	117 ± 26	144 ± 24	<0.06
Ammonia (μmol/L)	81 ± 9	91 ± 13	<0.3
Lactate (mmol/L)	13.1 ± 2.9	13.2 ± 2.2	<0.9
Albumin (g/dL)	3.7 ± 0.3	2.7 ± 0.1	<0.01
BUN (mg/dL)	12.0 ± 2.4	11.5 ± 2.7	<0.4
Creatinine (mg/dL)	1.6 ± 0.3	1.6 ± 0.3	<1.0
Group III			
Glucose (mg/dL)	141 ± 9	171 ± 11	<0.001
Ammonia (μmol/L)	173 ± 31	131 ± 15	<0.08
Lactate (mmol/L)	5.7 ± 1.1	5.6 ± 0.9	<0.9
Albumin (g/dL)	3.0 ± 0.1	2.6 ± 0.1	<0.00003
BUN (mg/dL)	38.5 ± 3.8	35.5 ± 3.2	<0.0004
Creatinine (mg/dL)	2.8 ± 0.3	2.2 ± 0.2	<0.00002

BAL = bioartificial liver; BUN = blood urea nitrogen.

It is clear that in the case of patients with FHF, for a liver support system to be effective, it has to arrest and/or reverse the rapid development of intracranial hypertension leading to brainstem herniation and death. If a system can achieve that, it will have a significant impact on outcome in this group of patients. Our data suggest that state-of-the-art critical care in addition to the BAL can result in significant reduction in ICP. It is unlikely that BAL treatment alone, in the absence of aggressive standard medical therapy, can arrest or reverse cerebral edema. At the same time, excellent medical therapy alone, historically, has not been successful in preventing permanent neurologic damage. Orthotopic liver transplantation is the only relatively novel therapeutic method that has had a significant impact on outcome in patients with FHF.[1] Therefore, a system that can arrest intracranial hypertension as a bridge to OLT ultimately will result in improved patient survival. The BAL, based on the preliminary data presented here, appears to be an effective bridge to OLT. Further appropriately controlled trials are being initiated to confirm this observation. Certainly the ultimate goal of a liver support treatment is to treat patients early enough and repeatedly to allow them to regenerate their livers and recover normal function. One patient in group 1 fell in this category, and she had full recovery without a transplant.

Use of the BAL in patients with PNF is justified only if the system can provide effective metabolic and physiologic support. The small number of patients treated with the BAL does not, at this stage, allow any meaningful analysis and conclusions. These patients need to be studied prospectively in a controlled trial. Assessing the efficacy of any support system in these patients is difficult. The presence of anesthetics and paralyzing agents makes neurologic assessment of the patients difficult. Rendering these patients, as well as patients with massive liver necrosis, anhepatic may make it possible to provide more effective support with the BAL. These issues, however, need to be tested and resolved in the experimental laboratory first.

We believe that the current BAL design can provide effective detoxifying, metabolic, and physiologic support because of a number of unique features that allow the design of a safe and effective system:

1. Use of collagen-coated microcarriers increases the surface area available for hepatocyte attachment and allows cell–matrix and cell–cell contacts, maintenance of normal cell polarity, and thus expression of differentiated function.
2. Use of large-pore fibers and high flow recirculation of plasma enhances greatly fluid convection and improves transfer of substrates and products to and from the hepatocytes. At the same time, it avoids whole blood perfusion with the risk of hemolysis,

Table 7. EFFECT OF BAL ON HEMATOLOGIC AND COAGULATION PARAMETERS

	Pre-BAL	Post-BAL	p
Group I			
Hematocrit (%)	31.1 ± 0.6	33.0 ± 0.7	<0.05
PT (sec)	21.5 ± 1.0	22.1 ± 0.7	<0.5
Platelets (1000/μL)	136 ± 14	116 ± 9	<0.01
Fibrinogen (mg/dL)	177 ± 11	124 ± 8	<0.00001
Factor V (%)	29.5 ± 3.5	18.1 ± 2.5	<0.00001
Group II			
Hematocrit (%)	29.8 ± 2.2	34.7 ± 1.7	<0.06
PT (sec)	21.6 ± 1.8	23.0 ± 2.1	<0.5
Platelets (1000/μL)	70 ± 32	59 ± 29	<0.08
Fibrinogen (mg/dL)	167 ± 29	121 ± 21	<0.06
Factor V (%)	19.5 ± 9.5	11.0 ± 1.0	<0.5
Group III			
Hematocrit (%)	29.3 ± 0.6	31.6 ± 0.5	<0.008
PT (sec)	22.6 ± 1.2	21.4 ± 0.7	<0.2
Platelets (1000/μL)	107 ± 16	105 ± 12	<0.8
Fibrinogen (mg/dL)	168 ± 20	142 ± 15	<0.007
Factor V (%)	28.0 ± 3.6	22.0 ± 3.0	<0.04

BAL = bioartificial liver; PT = prothrombin time.

clinically significant thrombocytopenia, and need for heparin administration. Heparinization of a patient with an ICP monitor in place carries a prohibitive risk.

3. Use of activated charcoal before the hepatocyte bioreactor, may provide a "protective" effect on the hepatocytes from the toxic plasma.
4. Placement of the BAL outside the primary plasma separation circuit, enhances the safety of the system.
5. Use of highly purified hepatocyte populations without antigen-presenting cells and cells rich in class II antigens reduces the immunogenicity of the xenogeneic cells.

We have carried out a phase I clinical trial to examine primarily the safety of the BAL. During the trial, based on clinical observations and parallel laboratory investigations, we addressed many issues dealing with streamlining the clinical process, obtaining meaningful data, and defining useful endpoints. We standardized clinical care protocols, the treatment system design, cell processing, and cell cryopreservation methods. Use of cryopreserved cells makes the system versatile and practical because it allows central cell harvesting and processing with shipping at treatment sites as needed. At a clinical site, cells can be processed easily and be ready for clinical use in approximately 2 to 3 hours.

The remarkable survival obtained in the group of patients with FHF is because of a combination of excellent clinical team skills and possibly the introduction of this innovative liver support technology. The role of the latter remains to be better defined in a controlled, randomized trial setting. For this or other liver support systems to play a meaningful role in the management of patients with exacerbations of underlying liver disease processes, such patients need to be treated early while they have a residual liver mass that can recover potentially after the acute precipitating event. These patients also will need to be treated for longer periods.

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Discussion

DR. BERNARD M. JAFFE (New Orleans, Louisiana): Dr. Cameron, Dr. Copeland. I was privileged to see this manuscript in its full form as all of you will be. Dr. Demetriou and his col-