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Fulminant liver failure (FHF) is associated with a high rate of mortality. Cell-based liver support systems have been developed for the treatment of FHF to prevent the development of potentially life-ending complications such as cerebral edema, intracranial hypertension, and brain herniation. Extracorporeal liver support systems, such as the bioartificial liver (BAL), contain hepatocytes intended to provide auxiliary hepatic function as a bridge to liver transplantation. Clinical trials indicate that the beneficial effects of BAL therapy include a reduction of cerebral edema and intracranial pressure, along with the possibility of spontaneous recovery in some cases. Enhancements to the BAL such as increasing the mass of viable and metabolically active hepatocytes are likely to be associated with greater efficacy in future clinical trials.

ABBREVIATIONS

FHF	Fulminant hepatic failure
BAL	Bioartificial liver
ICP	Intracranial pressure
SIRS	Systemic inflammatory response syndrome

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Introduction

Fulminant hepatic failure (FHF) develops in about 1000 patients annually in the United States and is the indication for liver transplantation in 5% to 10% of cases. Because it develops rapidly and often affects previously healthy individuals, FHF is considered to be one of the most severe forms of liver disease. Cerebral edema, a neurological manifestation of FHF, can lead to intracranial hypertension, herniation, and brain death within days to hours of clinical presentation.[1] Despite preferential allocation of livers to patients with FHF (i.e., United Network for Organ Sharing status 1), the survival rate in such patients after transplantation is significantly lower than that in patients with other causes of liver failure.[2]

The bioartificial liver (BAL) is one therapy developed to stabilize patients experiencing FHF. The BAL functions outside of the patient's body, similar to hemodialysis in the treatment of kidney failure. However, the BAL is a "hybrid" extracorporeal device in that it contains hepatocytes, most commonly of porcine or human origin, as a biological source of liver function.[3] A variety of names other than BAL have been used to describe hepatocyte-based extracorporeal liver support systems, including the hybrid liver support system, the modular extracorporeal liver system, and the extracorporeal liver assist device. BAL will be used preferentially in this article.

The list of BAL systems in clinical testing has grown steadily in recent years.[4-12] These systems and their unique features have been well summarized in several recent reviews.[3,13-15] The purpose of this article is 2-fold: (1) to expand on the role of BAL systems in the treatment of FHF based on the pathophysiology of FHF and recent observations using a porcine hepatocyte-based system and (2), based on these observations, to suggest ways in which current BAL systems can be improved.

Role of BAL in FHF

Clinical goals of BAL therapy in FHF are summarized in Table 1. A primary goal of the BAL in

Table 1 | **Clinical Goals of Bioartificial Liver Therapy**

Bridge to liver transplantation
Correct neurological manifestations of fulminant hepatic failure (FHF) (i.e., coma, cerebral edema, intracranial hypertension)
Potentiate spontaneous recovery of acutely injured liver
Improve survival of patients with FHF

FHF is to stabilize patients prior to liver transplantation. In doing so, stable FHF patients are more likely to tolerate the transplant procedure and experience improved long-term survival. Prevention of cerebral edema is an important secondary goal of BAL therapy, since cerebral edema can be an irreversible and life-ending neurological manifestation of FHF. If BAL treatment is successful, time becomes available to obtain a liver for transplantation or to allow the possibility of spontaneous recovery of the patient's acutely injured liver. The BAL may also be used to evaluate the extent of neurological injury and to assess the possibility of neurological recovery by patients with advanced FHF prior to liver transplantation. Potentially important hepatic functions of a BAL include a variety of biosynthetic activities such as gluconeogenesis and synthesis of proteins (e.g., albumin, clotting factors) and detoxification activities such as ureagenesis. BAL systems may also enhance recovery of the injured liver through modulation of humoral and molecular mechanisms of liver regeneration.[16] Quenching the systemic inflammatory release syndrome (SIRS) caused by the release of cytokines from necrotic liver tissue in FHF is another possible benefit of BAL therapy. Finally, auxiliary liver support may prevent the dysfunction of other extrahepatic organs such as renal dysfunction associated with FHF.

Hepatic Cerebral Edema

Several definitions of FHF have been proposed, each based on the development of mental status changes in a previously healthy individual (no history of chronic liver disease) days to weeks after the onset of acute liver failure and jaundice. Changes in mental status range from mild confusion to deep unresponsiveness and are scored from grade I to grade IV hepatic encephalopathy or coma, respectively. Hepatic cerebral edema is seen in higher grades of coma and can lead to a rise in intracranial pressure (ICP), cerebral herniation, and brain death. Cerebral edema is the cause of death in up to 50% of patients who die of FHF. Cerebral edema rarely develops in patients with chronic liver disease, suggesting a difference in the pathogenesis of encephalopathy in chronic liver disease compared to FHF. Alternatively, the brain has more time to adapt in the chronic situation and thus avoid edema formation. Bedside monitoring of ICP, usually by a catheter placed in the epidural, subdural, or intraparenchymal compartment, is the current gold standard for estimating the severity of cerebral edema in FHF. Hemorrhagic complications, including those related to the ICP catheter, may also occur in these coagulopathic patients, but should not preclude invasive monitoring.[17] In a recent report of 295 patients with acute liver failure, 25% survived without transplantation, 41% received a liver transplant (1-year survival 76%), and 34% died prior to transplantation.[18] Mortality of patients admitted in deep coma (grade III-IV) was higher than that of patients admitted in mild coma (grade 0-II) (68% vs. 27%, $P = 0.020$), indicating that outcome was influenced significantly by severity of neurological impairment. Other studies have shown that children are at especially high risk for developing neurological complications from FHF. In one recent report of 20 children with FHF, radiographically apparent cerebral edema was associated with 70% mortality, 15% severe neurological deficit, and 15% moderate deficit.[19]

The cause of cerebral edema in FHF remains unconfirmed, but is likely multifactorial, involving the accumulation of neurotoxic substances in the setting of reduced hepatic function.[1] Ammonia is one such substance whose arterial concentration has been shown to correlate with cerebral herniation in some, but not all, FHF patients.[20] In a study using moderate hypothermia to treat FHF, arterial ammonia and cerebral uptake of ammonia correlated with ICP.[21] In a more recent study, high cerebral ammonia uptake and cerebral glutamine efflux in patients with FHF were associated with an increased risk of subsequent fatal intracranial hyper-

tension.[22] For this reason, levels of arterial ammonia, along with ICP, are often used to evaluate the performance of BAL treatment in FHF.

At least 2 possible mechanisms may explain the harmful effects of ammonia to the brain. First, a high concentration of ammonia favors the conversion of α-ketoglutarate to glutamate by glutamate dehydrogenase. The subsequent conversion of glutamate to glutamine also requires ammonia and is catalyzed by glutamine synthetase, an enzyme identified within astrocytes of the brain.[23] Depletion of α-ketoglutarate, a citric acid cycle intermediate, leads to a decrease in the rate of formation of ATP in the brain and one possible mechanism of ammonia neurotoxicity. Second, the accumulation of brain glutamine appears to have a direct influence on the development of hepatic encephalopathy in FHF. Glutamine is an osmotically active substance that contributes to the formation of brain edema.[24] For example, swelling of astrocytes is a characteristic of acute hyperammonemia. Astrocyte swelling and cerebral edema are reduced by inhibitors of glutamine synthetase in animal models of hyperammonemia.[25] Human studies using magnetic resonance spectroscopy also indicate that the concentration of brain glutamine correlates with severity of encephalopathy during FHF.[26]

Lactate, another osmotically active waste substance produced in the brain of FHF patients, may also play a role in the formation of cerebral edema. Along with its apparent role in glutamine production and cerebral hyperemia, ammonia has been linked to the production of brain lactate. The rise in levels of brain lactate occurs independent of lactate levels in the blood, indicating an imbalance between anaerobic and aerobic metabolism in the brain. Increased lactate production in the brain of FHF patients may arise through an inhibitory effect of ammonia on pyruvate oxidation or by uncoupling of oxidative phosphorylation in cerebral mitochondria. A similar syndrome of impaired oxygen use is observed in peripheral tissues after major trauma and appears to be a cytokine-mediated process.[27] In the case of FHF, the injured liver may be the source of cytokine mediators, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and IL-6, and hepatectomy has been associated with temporary neurological improvement in some cases.[28]

A SIRS is observed in nearly 60% of acute liver failure patients.[29] The magnitude of SIRS correlated with the progression of encephalopathy in these patients.[29] Impaired autoregulation of cerebral blood flow beyond apparent metabolic needs is another characteristic of SIRS. Increased cerebral blood flow leads to increased cerebral blood volume, which contributes further to the development of intracranial hypertension. Cytokine mediators and ammonia may both have roles in the impaired cerebral blood flow of acute liver failure. Finally, with the development of intracranial hypertension, cerebral hypoperfusion and ischemia contribute to the rise in brain lactate that occurs with advanced cerebral edema and herniation.[30]

FHF and BAL: Mayo Experience

In support of the multifactorial theory of hepatic cerebral edema described above, we observed that treatment with a BAL system was associated with simultaneous reductions in arterial ammonia and ICP, along with improvement in biochemical and clinical measures of the systemic inflammatory response to FHF. Our experience consisted of 15 BAL treatments in 4 FHF patients with grade III to IV hepatic encephalopathy. Each patient was randomized to the treatment arm of a prospective phase II/III trial of the HepatAssist™ liver support system sponsored by Circe Biomedical (Lexington, MA).[12] The HepatAssist™ BAL used in this study contained about 75 g of cryopreserved porcine hepatocytes and an activated charcoal column through which the plasma fraction of blood was filtered. Treatments were performed daily for 6 h until the time of liver transplantation (patients 1, 2, and 4) or until spontaneous recovery without transplantation (patient 3). A standardized protocol that included measurement of ICP by intraparenchymal transducer (Camino NeuroCare, San Diego, CA) was used in the management of all FHF patients enrolled in this study at our center. The etiology of FHF included acetaminophen toxicity (patient 3) or was indeterminate (patients 1, 2, and 4).

BAL treatment was associated with a significant reduction in both arterial ammonia and ICP, as illustrated in Figure 1. The magnitude of reduction in ammonia concentration and ICP was greatest with 1st treatments, consistent with an advanced

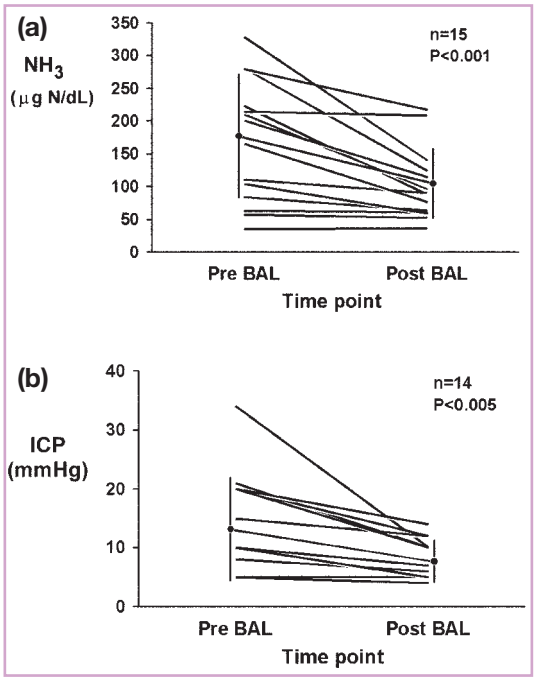

Figure 1. Changes in (a) arterial ammonia concentration and (b) intracranial pressure in patients with fulminant hepatic failure (FHF) immediately before (pre–bioartificial liver [pre-BAL]) and after (post-BAL) treatment with a porcine hepatocyte–based BAL. The decline in arterial ammonia concentration from pre-BAL to post-BAL was highly significant ($P < 0.001$) by paired *t* test. The decline in intracranial pressure (ICP) from pre-BAL to post-BAL was also significant ($P < 0.005$) by paired *t* test. ICP measurements were not possible during our 3rd patient's final BAL treatment, since his ICP transducer had been removed.

grade of encephalopathy at the time of enrollment. BAL therapy was also associated with a rise in cerebral perfusion pressure (defined as mean arterial pressure minus ICP), which increased on average (mean ± *SD*) from beginning (60 ± 14 mm Hg) to end (74 ± 10 mm Hg) of treatment ($P < 0.05$). Evidence of a decreased systemic inflammatory response to liver failure during BAL therapy included reductions in heart rate (mean = −6.8 beats per minute), core body temperature (mean = −0.4 °C), and plasma cytokine levels with each treatment. Average levels of the following cytokines dropped from pretreatment to end of therapy ($n = 4$): TNF-α, 220 to 19 pg/mL; IL-1β, 12 to < 5 pg/mL; IL-6, 7791 to 459 pg/mL; and IL-8, 625 to 88 pg/mL. The significance of reduction in cytokine levels with apparent improvement from BAL therapy remains unknown, but it may be related to the role of cytokines as mediators of SIRS and clearance during BAL therapy.

The clinical course of our 4th patient was most telling as to the benefits and limitations of BAL therapy using a treatment regime of 75 g of cryopreserved porcine hepatocytes (equivalent to less than 7% of estimated normal liver mass) daily for 6 h. Laboratory data (factor V, factor VII, and arterial ammonia) and ICP measurements associated with 7 BAL treatments are summarized in Table 2. The extent of acute injury and lack of regenerative capacity of this patient's native liver are demonstrated by a steady decline in the levels of human factor V and human factor VII during 7 days of BAL therapy. The gradual decline in post-BAL arterial ammonia levels after the 1st 4 BAL treatments indicates a beneficial effect of BAL therapy. However, the eventual rise in post-BAL arterial ammonia (beginning on day 5) and ICP (beginning on day 6) suggests that the extent of liver injury had progressed beyond a level that could be controlled indefinitely with daily BAL treatments. Our patient was fortunate to be successfully bridged to liver transplantation 8 days after status I listing.

Our 4th BAL case also illustrates that elevated levels of arterial ammonia (i.e., 329 μg N/dL on day 1) are not always associated with intracranial hypertension. This observation suggests that other factors must contribute to the development of cerebral edema. Of note, the rise in ICP experienced by our 4th BAL patient shortly before liver transplantation was associated with an elevated arterial ammonia level above 200 μg N/dL.

Our observations support the reports of others that BAL treatment is associated with a reduction in arterial ammonia, decreased ICP, and improvement in mental status.[5,7,9,28,31] Similar benefits have been reported with hypothermic treatment (33 °C to 35 °C) of uncontrolled intracranial hypertension in acute liver failure.[21] The average reduction in core body temperature that we observed during BAL therapy was only −0.4 °C, and therefore was insufficient to account for the improvements in neurological status by hypothermic means alone. It is unknown, but of importance, whether the beneficial effects of BAL therapy and hypothermic ther-

Table 2 | **Representative Data from a Patient with Fulminant Hepatic Failure of Undetermined Etiology Bridged to Liver Transplantation with the HepatAssist™ Bioartificial Liver System**

			Arterial Ammonia (μg N/dL)		
Study Day	Factor V[a] (% Activity)	Factor VII[a] (% Activity)	Pre-BAL	Post-BAL	ICP
1	42	7	329	140	< 5
2	29	6	210	96	< 5
3	25	4	111	90	< 5
4	18	2	104	59	< 5
5	20	7	166	76	< 5
6	16	3	201	114	< 10
7	11	2	214	208	10-20
8	(Post-LT) 78	82		13	< 5

The patient remained in grade 4 hepatic encephalopathy from the time of enrollment in the study (day 1) until liver transplantation (LT) on day 8. BAL = bioartificial liver, ICP = intracranial pressure.
a. Factor V and factor VII levels were determined by immunoassays specific for human proteins.

apy are additive in controlling cerebral edema during treatment of FHF.

FHF and BAL: Recent Clinical Trials

The 4 FHF patients described in the previous section were participants in a phase II/III multicenter, randomized study of the HepatAssist™ BAL. The results of that trial, the largest clinical investigation to test the efficacy of an extracorporeal liver-assist device, were recently published in abstract form.[12] A total of 171 patients were studied, including 147 with FHF (the remaining 24 patients had primary nonfunction after liver transplantation). The 30-day survival rate of FHF patients was increased by 14% with BAL treatment (control, 59% vs. BAL, 73%), although this difference did not reach statistical significance. A significant benefit of HepatAssist™ BAL therapy was reached when confounding factors such as time to transplantation, stage of encephalopathy, and etiology of FHF were accounted for in a covariate, time-dependent proportional hazards model. According to this model, the greatest benefit of BAL therapy was observed in acetaminophen-induced FHF (P = 0.018) and FHF patients developing encephalopathy within 2 weeks of jaundice (P = 0.05). Another important finding of this study was the absence of porcine endogenous retrovirus infection in any study patient exposed to porcine hepatocytes. Approximately 50% of patients subsequently underwent liver transplantation and immunosuppressive therapy.

The use of a BAL system incorporating primary hepatocytes isolated from discarded human livers (not acceptable for human liver transplantation) to treat FHF has been reported recently.[11] Human hepatocytes are being considered because of their favorable metabolic profiles with respect to human therapy and in response to regulatory and infectious concerns with regard to the use of animal tissues in human studies. Along with a large cell mass of approximately 500 g of human hepatocytes, this group's modular system, called the modular extracorporeal liver system, is capable of other functions such as dialysis and detoxification. These other functions are accomplished by adding modules in series or in parallel to the extracorporeal circuit. A favorable response was reported in an initial experience of 2 cases using this truly "hybrid" BAL system.[11]

Millis et al.[10] recently reported the results of a phase I trial of the extracorporeal liver assist device (Vitagen, La Jolla, CA) containing approximately 400 g of C3A (immortalized cell line obtained from human hepatoblastoma) cells for the treatment of patients with FHF. Along with acceptable safety data, a trend toward improved survival was observed in this controlled study of FHF patients listed for transplantation (30-day survival: 10/12, 83% vs. 3/7, 43%, P = 0.12). The C3A cell line of-

fers the advantage of using human cells with improved growth profiles under ex vivo culture conditions compared to primary hepatocytes obtained from discarded human livers. However, metabolic profiles of liver tumor cells are incomplete, including the C3A cell line, and spread of tumor cells from device to patients is a theoretical possibility.

Areas for Improvement

All 3 BAL systems discussed in the previous section employed cartridges of hollow fibers to separate the patient's blood from hepatocytes in the device. The hollow fibers were made of porous material and allowed the transfer of molecular wastes (i.e., ammonia, unconjugated bilirubin) and metabolic products (i.e., urea, conjugated bilirubin) to and from the cultured hepatocytes, respectively. The pores prevented cellular passage and served as immunological barriers to cell-mediated rejection, thus eliminating the need for immunosuppressive medications during BAL therapy.

Despite the advantages of hollow fiber systems, significant problems still exist. These problems include (1) premature death of hepatocytes under ex vivo conditions, (2) inadequate mass of hepatocytes in the BAL cartridge, and (3) convenience with respect to an ample supply of cells that can be easily made available at the time of treatment. Problems 1 and 2 are likely due to environmental factors and nutrient limitations that exist within the extracorporeal BAL system. These 2 problems may be best addressed by the modular extracorporeal liver system; however, reliance on hepatocytes from discarded human livers alone may prove problematic. Alternatively, these problems have been addressed by using hepatocytes from a transformed liver cell line. However, as pointed out earlier with the C3A cell line, metabolic profiles of liver tumor cells are incomplete and lead to limitations of BAL performance.

With regard to hepatocyte mass, the number of hepatocytes required to support a patient in FHF is unknown and probably varies with patient size and severity of illness. The mass of hepatocytes reported in clinical BAL studies has ranged from 50 g to 680 g, assuming 1 to 2×10^8 hepatocytes/g.[8,32] Based on the experience of hepatic resections in humans, approximately 150 to 400 g of viable, normal-functioning hepatocytes are needed to support normal hepatic function in an adult.[33] We would favor a device loaded with at least 500 g of viable hepatocytes to allow for cell death over time in the BAL device. The clinical success of BAL therapy reported using porcine hepatocytes,[8] human hepatocytes,[11] and C3A cells[10] would support this recommendation.

Pros and cons exist for the use of all cell types proposed to date in a BAL, including pig hepatocytes, human hepatocytes from discarded livers (not used for transplantation), human stem cells, human liver tumor cells, and a wide variety of other immortalized human cell lines.[34] The potential risks and benefits have been reviewed in detail elsewhere.[35] Based on current risk-to-benefit profiles, we prefer the use of primary hepatocytes (either of porcine or human origin) as the source of metabolic function in a BAL. A safe, fully functional line of immortalized human hepatocytes would be ideal but does not exist at this time.

For now, we feel that primary hepatocytes and modern cryopreservation techniques can be used to satisfactorily address the problems of premature death, cell mass, and convenience described above. Premature death of hepatocytes in a BAL appears to involve both necrosis and apoptosis. These processes are initiated at the time of hepatocyte isolation and continue until hepatocytes are restored to a satisfactory culture environment. Necrosis and apoptosis continue if the culture environment is unsatisfactory. Numerous investigators have shown that a supporting biomatrix, such as collagen gel, can be used to improve viability and function of hepatocytes in culture. More recently, it has been recognized that liver cells are capable of producing their own biomatrix in vitro and forming aggregates, sometimes termed hepatocyte spheroids, with high viability and stabile function.[36] Spheroid-based BAL systems have been proposed,[37] although they have not yet been reported in clinical trials.

Cryopreservation is an important process that will contribute to the eventual success of BAL therapy. Cryopreservation allows primary hepatocytes to be isolated at convenient times and stored by clinical sites prior to emergency use. Safety tests can be performed easily on cryopreserved samples of cells. Furthermore, cryopreservation avoids the costs and contamination risk of long-term hepatocyte culture. Cryopreservation is associated with caspase

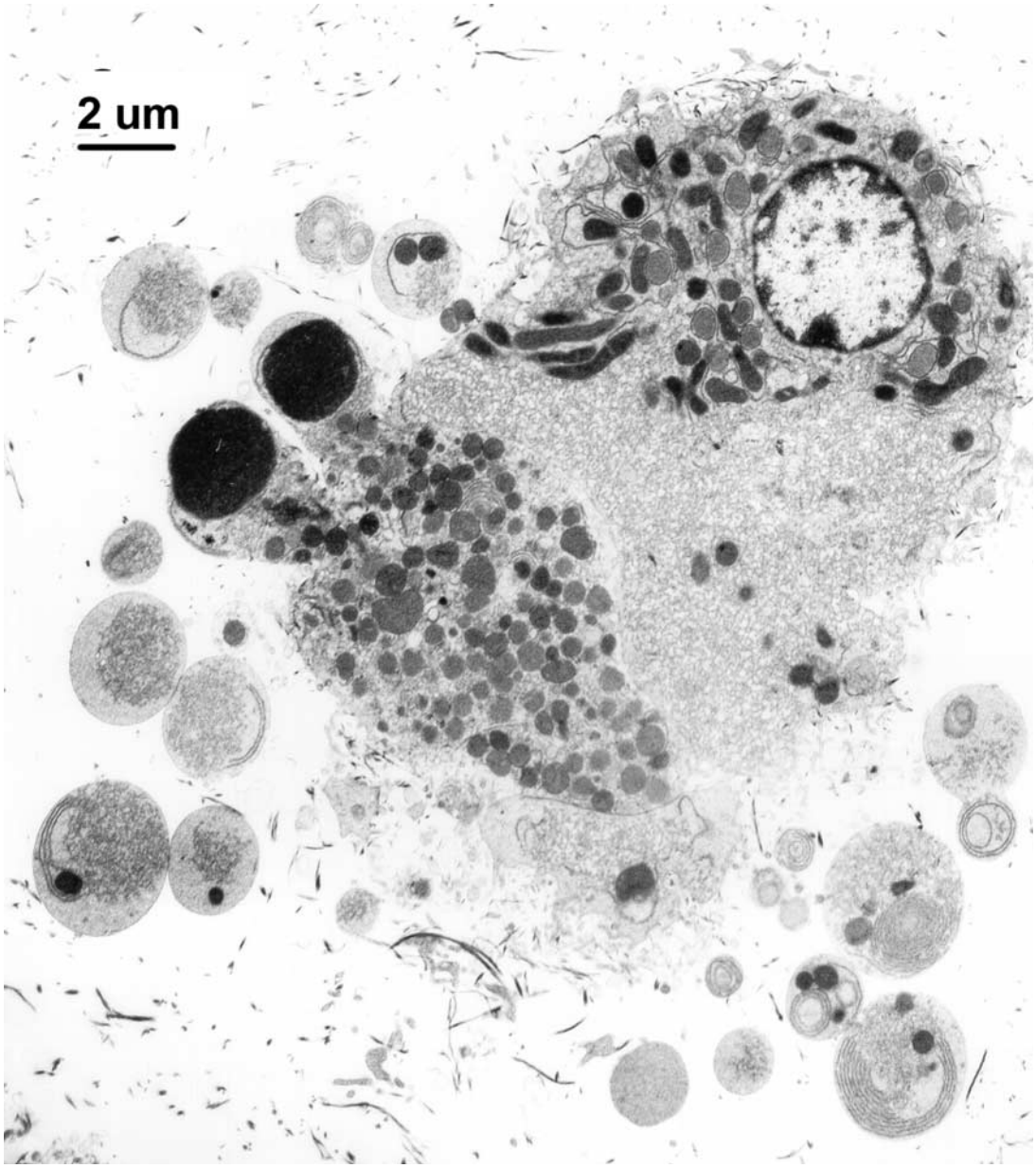

Figure 2. Normal hepatocyte (upper right) and apoptotic cell (lower left) after hepatocyte isolation, cryopreservation storage at –80 °C, and 6 h of culture. Apoptotic features include chromatin condensation in both nuclei of this binucleated cell and division of the cytoplasm into multiple organelle bodies.

activation and apoptotic cell death, however, as illustrated in Figure 2. Apoptosis, which accounts for more than 50% of cell death after cryopreservation, can be reduced significantly by adding cytoprotective agents such as the caspase inhibitor ZVAD-fmk to the cryopreservation media.[38] Newer generation caspase inhibitors will avoid the potential toxic side effects of ZVAD-fmk and are expected to further reduce the apoptotic injury incurred by hepatocytes during cryopreservation.

Spheroid formation (discussed above) is associated with improved viability and metabolic activity of

Table 3 **Albumin Production (μg/h/10^8 Cells)**

Culture	Spheroid	Collagen Gel		Monolayer	
Day[a]	Mean ± *SD*	Mean ± *SD*	*P* Value[b]	Mean ± *SD*	*P* Value[b]
1	7.6 ± 0.5	8.2 ± 0.6	> 0.05	7.2 ± 0.7	> 0.05
3	21.8 ± 3.8	12.1 ± 2.0	< 0.001	10.1 ± 2.9	< 0.001
5	32.7 ± 3.0	14.7 ± 8.3	< 0.001	4.1 ± 1.0	< 0.001

a. Day in culture after 7 days of cryopreservation storage at –80 °C. Cells were grown under the following conditions: suspension culture after spheroid formation (spheroid), entrapped collagen gels (collagen gel), or as monolayers on tissue culture plastic (monolayer). An initial cell concentration of 5×10^5 cryopreserved porcine hepatocytes per milliliter of culture medium was used in each condition.

b. *P* value versus spheroid culture group on same day of culture by unpaired *t* test.

primary hepatocytes after cryopreservation (Table 3). In fact, spheroids formed from cryopreserved porcine hepatocytes had significantly greater metabolic activity than an equal number of freshly isolated porcine hepatocytes in monolayer culture on day 3 (32 ± 9 vs. 9 ± 4 μg/h/10^8 cells, $P < 0.05$) and day 5 (59 ± 13 vs. 12 ± 5 μg/h/10^8 cells, $P < 0.01$). These observations suggest that a spheroid-based BAL system using cryopreserved primary hepatocytes may address the 3 problems of premature death, cell mass, and convenience. In addition, continuous clinical operation of a spheroid-based BAL may be possible based on preliminary evidence of rising hepatic function by cryopreserved hepatocytes in spheroid culture (Table 3).

Summary

FHF is a dangerous form of liver disease due to its often-rapid course and associated complications that include cerebral edema, intracranial hypertension, and brain herniation. The BAL has been proposed as a treatment for FHF by providing auxiliary hepatic function to prevent the development of these potentially life-ending complications. Clinical data indicate that the beneficial effects of BAL therapy include a reduction of cerebral edema and ICP, along with the possibility of spontaneous recovery in some cases. Improvements to the BAL, such as increasing the mass of viable and metabolically active hepatocytes, are likely to be associated with greater efficacy in future clinical trials.

Acknowledgments

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