Extracorporeal Cellular Liver Assisted Devices

67

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Key Concepts

- Currently available hepatic assist devices have limited studies in acute liver failure
- Complex physiologic liver functions cannot be emulated despite advances in technology
- Extracorporeal devices may be of benefit in the subset of patients with acute liver failure
- Better randomized-controlled trials with strict inclusion and exclusion criteria as well as high power will need to be undertaken to fully understand the utility of these devices.

67.1 Introduction

Patients with acute liver failure (ALF) require liver transplant for definitive therapy unless the liver is able to regenerate. Many patients however may not survive until a suitable liver is available or may not be candidates for transplant. In addition, patients with long-standing liver disease may undergo sudden onset of decline and acute liver failure which is termed acute on chronic liver failure (ACLF) which may not be amenable to standard medical therapy [1].

Therefore, other treatment modalities that may reduce morbidity and mortality and perhaps serve as a bridge to transplantation may be an additional option. One particular avenue that has been investigated are the hepatic assist devices. Such devices aim to temporarily assume metabolic and excretory functions of the liver and thereby allow stabilization of patients who await transplant. This chapter will focus on the bioartificial devices that incorporate liver cells to accomplish this task [2–6].

67.2 Bioartificial Liver Support (BALS)

The BALS are based on the concept of dialysis with cell-based techniques that utilize animal or human liver cells to replace all of the intricate detoxification, synthetic (proteins and clotting factors) regulatory (hormones) and immunologic functions of the liver. This is accomplished by incorporating a bioreactor into the extracorporeal circuit that consists of hepatocytes. These cells are cultured in a 3-D matrix and surrounded by fibers that allow capillary perfusion. Oxygen and carbon dioxide are exchanged and glucose is supplied to mimic human physiology [7].

However, the limitations of producing such devices lies in the complexity of the liver functions themselves. The main issues that arise in the development of these devices is the selection of the source of liver cells and the stabilization of normal physiologic function with the artificial bioreactors [8].

The ideal bioartificial liver assist device would use human hepatocytes to closely mimic human physiology. However, a good-quality source of a large number of these cells is not currently available to accomplish this task. Most human hepatocytes would come from unused cadavers or from partial hepatectomy specimens which are uncommon. The quality of these specimens is inadequate as the better-quality ones are usually used for liver transplantation.

Currently the two cell sources that have been used in human clinical trials for bioartificial liver support systems are the human hepatoblastoma cell line, HepG2/C3A and primary hepatocytes from healthy pig livers [9].

C3A cells have numerous proteins that produce anti-inflammatory effects. They express anti-apoptotic and

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anti-oxidative mechanisms that decrease hepatocellular injury. They also express growth factors that are involved in regeneration of hepatocytes following acute phase response to injury [10].

67.3 Extracorporeal Liver Assist Device

The Extracorporeal Liver Assist Device (ELAD®) is a bioartificial liver assist device. Cartridges containing hollow fibers filled with human hepatoblastoma cell lines, HepG2/C3A, are employed in this device. These cells have hepatocyte properties, such as a functional CYP450 enzyme system and the ability to produce liver-specific proteins. They have shown a higher level of albumin secretion as well. It employs the use of whole blood for perfusion and can be continued for long periods of time [11]. These cell lines are originally from human liver tumor and therefore there is a theoretical risk that tumor dissemination can occur. However, there have been no reports of transmission of cancer thus far in the patients treated with these cells [12].

67.3.1 Extracorporeal Circuit

The system is connected in a closed circuit via venous access gained by placement of a double-lumen dialysis catheter in either the internal jugular or femoral vein. Four ELAD cartridges are used in this circuit to give a hepatocyte mass of 400 g. These cartridges are composed of thousands of hollow fibers that are semipermeable. The C3A cells are grown in the extracapillary space around these hollow fibers. Patient's blood is ultra-filtrated to isolated plasma ultra-filtrate. This plasma is then pumped through these cartridges via a standard dialysis pump at a rate of 150–200 mL/min. Anticoagulation is achieved using heparin with an initial bolus and then continuous infusion to achieve an activated clotting time of 200–250 s. An oxygenator is used to ensure adequate oxygen supply to the cells. Negative pressure is applied across the membranes to achieve an ultra-filtrate before being returned to the patient [13, 14]. A schematic representation can be seen in Fig. 67.1.

67.3.2 Studies

A phase 1 trial was performed in 11 patients most of which had acute liver failure. Improvement in mental status occurred in 8 of the 11 patients. Of the group, 4 were successfully bridged to OLT and six patients died before OLT while 1 survived without OLT [15].

The pilot ELAD study enrolled 24 patients with acute liver failure, 17 of whom had been considered to have potentially recoverable disease (Group 1) and 7 that had been listed for transplant (Group 2). Each of these subsets were then randomly assigned to ELAD vs. control (standard medical therapy). The median period of treatment was 72 h. There were no issues with biocompatibility and patients remained hemodynamically stable on the device. Six patients in Group 1 deteriorated and were placed on the waiting list for OLT. In patients treated with ELAD, ammonia, bilirubin and hepatic encephalopathy improved when compared to standard medical treatment. There was no survival benefit in either group (survival rates were 78% and 75% in Group 1 and 33% and 25% in Group 2) for patients treated with and without ELAD, respectively [14].

In a follow-up study in which ultra-filtrate was used instead of whole blood, Millis et al., studied [5] patients with ALF who were bridged to transplant using ELAD. The patients tolerated the treatment well and the clinical course for the treated patients appeared to be stabilized. The 30-day survival rate was 75%. Other parameters that showed

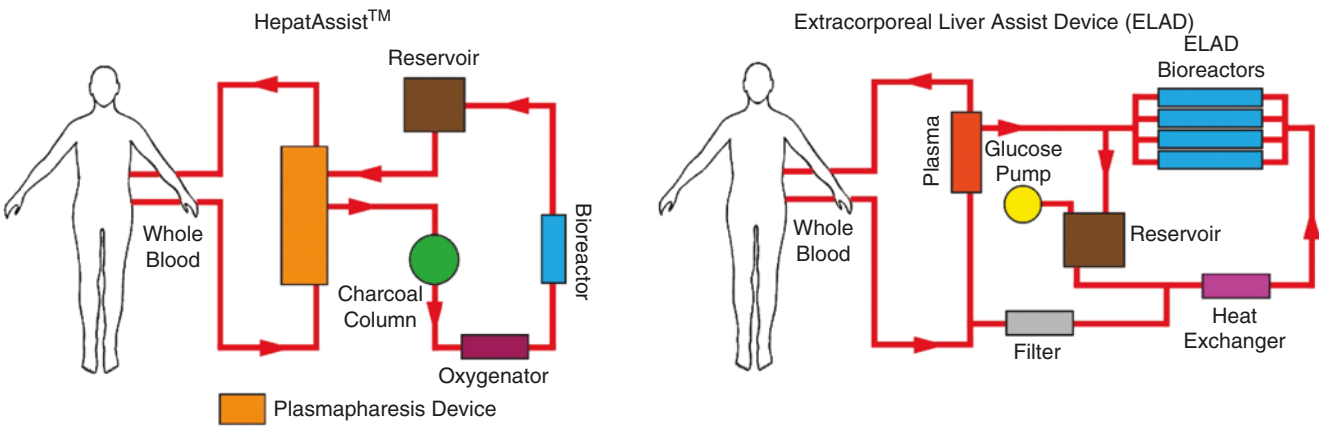

Fig. 67.1 Schematic extracorporeal circuit for HepatAssist and ELAD devices

improvement included mean arterial pressures, cerebral perfusion pressures, and reduction in cardiovascular and ventilator support [13].

An open-label randomized controlled trial was conducted in two Chinese Centers to evaluate ELAD in patients with chronic hepatitis B and C infection. A total of 49 patients were enrolled of which 32 were treated with ELAD. The 28-day transplant-free survival was 47% in the control group vs. 81% in the ELAD group ($p = 0.022$). Total bilirubin level decreased by 25% in the ELAD group vs. 37% increase in the control group ($p < 0.001$). Thrombocytopenia occurred in a majority of patients however with a mean drop in counts of 28% from baseline. However, the counts recovered within 5 days of ELAD discontinuation [16].

More recently, a randomized multi-center clinical trial using ELAD for patients with severe alcoholic hepatitis was published (VTI-208 [Assess Safety and Efficacy of ELAD (Extracorporeal Liver Assist System) in Subjects with Alcohol-Induced Liver Failure]). The study population was defined as adults ≥18 years of age with last drink within 6 weeks of rapid onset of jaundice (serum bilirubin ≥8 mg/dL) and coagulopathy (Maddrey's DF ≥ 32) and Model for End-Stage Liver Disease (MELD) score ≤35. Patients were randomized to ELAD for 3–5 days plus standard of care vs. standard of care alone. Unfortunately, after a minimum follow-up of 91 days, there was no significant difference in overall survival between groups. However, in a pre-specified analysis in patients with MELD < 28 there was a trend toward higher survival at 91 days (68.6% vs. 53.6%; $p = 0.08$). Using regression analysis, high creatinine and INR were associated with negative outcomes. Therefore a new trial investigating the potential benefit of ELAD in younger patients with sufficient renal function and less severe coagulopathy was done in 2018 [10]. Unfortunately, the study failed to meet the primary endpoint of overall survival through 91 days using the Kaplan Meier statistical method. The secondary endpoint of proportion of survivors at study day 91 was also not statistically different between study groups. There were no differences between groups regarding safety and tolerability of the treatment. Therefore, at this time, ELAD cannot be approved for management of either ALF or ACLF until further studies are completed.

Smaller studies have been presented regarding the anti-inflammatory effects of the C3A cells based on data from VTL-208. Plasma from cohorts with severe alcoholic hepatitis that met inclusion criteria were assayed for a variety of inflammatory markers. When compared to controls, levels of procalcitonin and ferritin were significantly reduced in ELAD patients. Levels of Interleukin-1 receptor antagonist (IL-1Ra) which reduces inflammation was higher in the ELAD arm as well. This may suggest that HepG2/C3A cells release products that dampen the inflammatory response [17].

67.4 HepatAssist™

HepatAssist (Alliqua Inc., Langhorne, PA, USA) is made from porcine hepatocytes that are contained within a hollow fiber bioreactor [18]. It uses plasma that is obtained from the patient's blood that is separated via plasmapheresis and then passed through the circuit containing porcine hepatocytes.

67.4.1 Extracorporeal Circuit

The system includes a perfusion pump, a charcoal column, an oxygenator, and custom tubing that connects the various components to a plasmapheresis machine [19]. During its use, plasmapheresis is performed via a double-lumen catheter. The plasma is pumped into the HepatAssist device and continuously circulates the plasma through the hollow fiber reactor. The charcoal provides detoxification and diminishes the toxin load applied to the hepatocytes. The membrane oxygenator ensures adequate oxygen supply. The plasma flows through the hollow fibers that are surrounded by the porcine hepatocytes. There are $5–7 \times 10^9$ cryopreserved porcine hepatocytes attached to beads which are inoculated into the extrafiber compartment. The pore size is small enough to prevent cell debris from passing into the patient [18]. This can be seen in Fig. 67.1. An improved version, HepatAssist-2 was created with an increased cell mass of 15×10^9 hepatocytes.

67.4.2 Studies

The largest, randomized, multicenter trial involving HepatAssist involved 171 patients with ALF or primary non-function after liver transplantation. The patients in the HepatAssist group underwent 6 h of treatment with the number of treatments ranging from 1 to 9 (mean 2.9) per patient. The 30-day survival was 71% versus 62% ($P = 0.26$) for the HepatAssist group compared to standard medical therapy, respectively. The study was stopped prematurely due to futility in the safety interim analysis. Though there was no survival benefit in the overall cohort, survival in the subgroup of patients with fulminant or sub-fulminant hepatic failure was significantly higher in the HepatAssist group compared with control with a 44% reduction in mortality ($P = 0.048$). Serum bilirubin had a statistically significant reduction in patients receiving HepatAssist, however there were no changes in encephalopathy, hemodynamics, or other lab values. In the subgroup of patients with acute liver failure, there was a significant difference in the time to death within the first 30 days compared to the control group ($p = 0.009$). No zoonosis or immune reactions were reported though this still remains a

concern [18]. Despite the survival benefit identified in a post hoc subgroup analysis, the FDA did not approve the HepatAssist device.

67.5 Modular Extracorporeal Liver Support System (MELS)

MELS was Initially developed by Gerlach et al. in Berlin, Germany using a unique multi-compartment bioreactor unit (CellModule) and detoxification unit (DetoxModule) using the concept of single-pass albumin dialysis for removing albumin-bound toxins [20].

67.5.1 Extracorporeal Circuit

The bioreactor contains three interwoven hollow-fiber membranes aimed at reproducing the liver vascular network [21]. Up to 600 g of porcine hepatocytes or human hepatocytes are inoculated into the extracapillary space. The patient's plasma is separated from the blood via plasma filter and recirculated through the hollow fibers at 200–250 cm^3/min. The device can combine different extracorporeal units that can be personalized to patient needs using either single pass albumin dialysis (SPAD) or continuous veno-venous hemodialysis (CVVHD) [22]. The first system used primary porcine cells from pigs. Later MELS became the only system that used primary human hepatocytes isolated from donor livers as well as porcine hepatocytes.

67.5.2 Studies

In a phase 1 clinical study published in 2003 by Sauer et al., eight patients with acute liver failure were treated with MELS continuously for 8–46 h. All patients were successfully bridged to OLT with 100% 3-year survival. More importantly, the therapy was well tolerated [20].

Due to rising concern for xenogenic infections using porcine cells, primary human cells were isolated from discarded donor organs as an alternative source. Cells from 54 human livers were isolated from grafts that were not suitable for transplant due to a variety of reasons (steatosis, cirrhosis, and fibrosis) [23]. Prepared bioreactors using these cells were then used to treat 8 patients with liver failure for 7–144 h. Once again, no adverse events were observed. Six of these patients were bridged successfully to transplant and the other two were not due to active alcohol consumption. In all patients, neurological and coagulation status improved during the treatment [20].

67.6 Bioartificial Liver Support System (BLSS)

The BLSS system was first developed at the University of Pittsburgh and employed the use of semipermeable cellulose acetate hollow fibers containing porcine hepatocytes. It uses whole blood perfusion instead of plasma [24].

67.6.1 Extracorporeal Circuit

The BLSS consists of a blood pump, heat exchanger to control the temperature of the blood being exchanged, oxygenator, and a bioreactor. The bioreactor contains hollow fibers with cellulose acetate membranes with a 100 kDa size cutoff. About 70–100 g of primary porcine hepatocytes are harvested and infused into the extraluminal space of the bioreactor. After loading these hepatocytes, the bioreactor is kept under physiologic conditions in an incubator prior to use with the patient. Oxygenation and pH control is maintained with the use of mass flow controllers [25].

67.6.2 Studies

The first clinical use of BLSS was in a 41-year old patient with acute liver failure. After treatment with BLSS the patient's ammonia, total bilirubin and lactate all improved. In addition, the coagulation function and clinical symptoms also improved and the patient was removed from the treatment [25].

A phase 1 clinical trial was then done on 4 patients with different etiologies of acute liver failure including acetaminophen toxicity, Wilson's disease, acute alcoholic hepatitis and chemotherapy. The mean ammonia and total bilirubin levels decreased after treatment (33% and 6% respectively). Renal and neurologic function did not improve however and survival data was not mentioned. All patients tolerated the system well [26].

67.7 Conclusions

Though orthotopic liver transplantation is the gold standard therapy for treating acute liver failure, there have been dramatic advances in liver support strategies to cope with the shortage in available donor organs. As outlined above and in Table 67.1, bio-artificial extracorporeal cellular assist devices have shown some promise. However, due to difficulty in creating a well-structured randomized-controlled trial with adequate power is difficult in this diverse population. In addition, standard medical therapy varies from institution to institution and therefore broad applicability is

Table 67.1 Bioartificial liver assist devices study outcomes

Study	N	Device	Cell type	Outcome
VTI-208, 2015 [10]	203	ELAD	Human (cultured C3A)	No survival benefit at 90 days
VTL-308, 2018	151	ELAD	Human (cultured C3A)	No survival benefit at 90 days
Ellis et al. [14]	24	ELAD	Human (cultured C3A)	No survival benefit
Demetriou et al. [18]	171	HepatAssist	Porcine	No survival benefit at 30 days
Sauer et al. [20, 22]	8	MELS	Porcine	100% Survival benefit as bridge to transplant
Mazariegos et al. [26]	4	BLSS	Porcine	No survival data

difficult. Treatment with these devices is usually followed by urgent OLT and therefore the 30-day survival is largely influenced by the outcomes of the OLT. The published results point towards the need for new trials with improvements in the system. The obvious limitations of these support systems are the membranes used for appropriate exchange and the lack of complete physiologic function. The government has yet to approve any of these bioartificial systems for this reason.

In addition, new approaches not using extracorporeal devices such as hepatocyte transplantation, repopulation of decellularized livers, organogenesis and stem cell transplant appear to be appealing. Further research is in need in order to improve survival of this difficult to manage population.

Self Study

Question

1. Which statement is true?
   (a) ELAD employs the use of porcine hepatocytes
   (b) MELS employs the use of only porcine hepatocytes
   (c) HepatAssist employs the use of porcine hepatocytes
   (d) BLSS uses plasma for exchange

Answer

1. Which statement is true?
   (a) ELAD uses human hepatoblastoma cells not porcine hepatocytes
   (b) MELS uses both porcine and human hepatocytes
   (c) CORRECT ANSWER. HepatAssist uses porcine hepatocytes
   (d) BLSS uses whole blood for exchange not plasma

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