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GUIDELINES FOR CLINICAL PRACTICE

Future of bioartificial liver support

Robert AFM Chamuleau

Robert AFM Chamuleau, Department of Hepatology, Academic Medical Center, University of Amsterdam, Meibergdreef 69-71, 1105 BK, Amsterdam, The Netherlands
Author contributions: Chamuleau RAFM contributed solely to this paper.
Correspondence to: Robert AFM Chamuleau, MD, PhD, Department of Hepatology, Academic Medical Center, S-Building, Floor 1, Room 166, Meibergdreef 69-71, 1105 BK, Amsterdam, The Netherlands. r.a.chamuleau@amc.uva.nl
Telephone: +31-20-5666832 Fax: +31-20-5669190
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Abstract

Many different artificial liver support systems (biological and non-biological) have been developed, tested pre-clinically and some have been applied in clinical trials. Based on theoretical considerations a biological artificial liver (BAL) should be preferred above the non-biological ones. However, clinical application of the BAL is still experimental. Here we try to analyze which hurdles have to be taken before the BAL will become standard equipment in the intensive care unit for patients with acute liver failure or acute deterioration of chronic liver disease.

Key words: Acute liver failure; Bioartificial liver; Liver transplantation; Cell transplantation; Tissue engineering; Xenotransplantation

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INTRODUCTION

Nowadays intensive care doctors have many artificial devices to support their patients with failing organs. They possess different types of hemodialysis devices, artificial ventilation, artificial heart, aortic balloon pumping, blood oxygenators, heart-lung machines and are able to apply total parenteral nutrition in the patient with short bowel syndrome. However, the patient with acute liver failure (ALF) is still a major challenge[1].

ALF is a devastating clinical syndrome with a high mortality (60%-80%, depending on the cause and the experience of the clinical centre) with most frequent causes of death being brain edema, SIRS (systemic inflammatory response syndrome) and multiple organ failure (MOF). Emergency whole or partial liver transplantation (orthotopic (OLT) or auxiliary) is the only life-saving therapy.

Many attempts have been made to develop artificial liver support devices (ALSD): non-biological ones such as hemodialysis, charcoal hemoperfusion, selective plasma filtration, plasma exchange, hemo-diadsorption, albumin dialysis and biological ones such as whole liver perfusion, liver cell transplantation and bioartificial liver support.

The status of ALSDs has been the subject of many reviews, at least one a year, since 2001[2-14]. It does not seem wise to repeat their contents in this editorial and the reader is referred to the publications for global and/or detailed information. From reading them, at least one common conclusion emerges: devices that only support the failing detoxification function of the severely diseased liver are not sufficient to save the lives of ALF patients. It is generally accepted that the syndrome of ALF is not only determined by failing hepatic detoxification, but also by failing hepatic synthetic and regulatory function. This is also one of the conclusions of a workshop on ALF held in the USA in 2008[1].

The purpose of this editorial is, however, to analyze the critical issues that have to be solved in the near future.

CRITICAL ISSUES

What can we expect from cell or even organ-based ALSDs?

Whole animal liver perfusion as an ALSD seems to be a logic approach. There is some experience in a few case reports[15], but it has never been accepted as a common treatment, because of its complexity and its important xenotransplantation-related problems.

There are a few case reports concerning the more simple technique of liver cell transplantation (LCTX)[16] as a treatment for ALF. LCTX has at least 2 important drawbacks: (1) the availability of sufficient amounts of fully differentiated human liver cells; (2) the so far unsolved problem of transplanting large amounts (at least 10% of the normal parenchymal mass) of cells where there is adequate blood supply.

Ideally, a tissue-engineered transplantable liver should be the final solution. Such a bioengineered liver (BEL) should resemble the native liver as much as possible. This means a composite of parenchymal and non-parenchymal liver cells in a sponge-like configuration in which a vascular system provides, by direct plasma contact, oxygen and nutrients to the liver cells and which is equipped with a biliary outflow system. Ideally this BEL has to be connected to the splanchnic circulation (inflow tract), the caval vein system (outflow tract) and the intestine (biliary tract).

At present, such a BEL is only in a very preliminary and experimental phase[17-22] and, as second best to liver transplantation, patients have to be treated by one of the existing bioartificial livers (BALs) that can only be connected outside the body to the patient's systemic blood circulation

A BAL is defined as a bioreactor charged with liver cells that is connected outside the body to the blood or plasma circulation of the patient. Since a BAL supports both the failing detoxification and the failing synthetic and regulatory function of the diseased liver, it should have a beneficial effect on the degree of hepatic coma and the severity of MOF and, last but not least, on survival of ALF patients and preferably also of patients with acute on chronic liver disease (AoCLD).

In general, 4 types of BAL bioreactors can be distinguished: hollow fiber; flat plate and monolayer; perfused beds/scaffolds; encapsulation/suspension. Every system has its pros and cons. For details see Allen *et al*[2].

To prove their right to belong to the standard equipment of an intensive care unit BALs need to be validated in randomized, controlled clinical trials. Several questions have arisen as to whether pre-clinical research on BALs has been sufficient to justify their clinical application.

How good are results in experimental animals?

In general, the answer is positive. In many different models of ALF several BALs based on animal liver cells have shown to prolong survival significantly in comparison to standard treatment[23-33].

Can it contain a sufficient mass of parenchymal liver cells?

It is generally accepted (based on safe surgical resections) that survival is possible with a minimum of 20% of liver mass with optimal functionality. Assuming that the ALF patient still has some residual functioning liver mass, a BAL should contain at least 15% of liver mass. However, the reality is that isolated liver cells in a bioreactor do not have optimal functionality, so more than 15% (preferably 20%-30%) of liver mass will be required[34].

Furthermore, it is well known that parenchymal liver cells function at best in a 3-dimensional (3D) configuration. In addition their functionality increases when they are co-cultured with non-parenchymal cells[35] For these reasons, the ideal BAL should contain at least a mixture of well-differentiated liver cells in a 3D configuration at a mass of at least 20% of the normal liver (200 g cells in 1 kg of liver). Vital Therapies ELAD® (Extracorporeal Liver Assist Device) and Hep-Art AMC-BAL have this capacity.

How is bi-directional mass transport of oxygen, carbon dioxide, nutrients and liver cell products best guaranteed?

In BAL devices, bi-directional mass transfer is needed to provide nutrients to sustain cell viability and allow export of therapeutic cell products. Although most device designs address this, there are important limitations involving the use of semi-permeable membranes as a barrier between plasma and the bio-component. Bioreactors in which direct contact between plasma and the liver cells is guaranteed or those using semi-permeable membranes with high porosity are preferred.

In addition, liver cells need sufficient oxygen supply to function optimally[36]. The amount of oxygen actually dissolved in plasma is insufficient in this respect. Therefore, the cells in the bioreactor should see either full blood (with many problems such as hemolysis, clotting and platelet loss) or plasma with an extra oxygen carrier such as fluorocarbons[37] or locally supplied oxygen by oxygen capillaries interwoven with the cell containing hollow fibers (Modular Extracorporeal Liver Support)[38] or matrix (AMC-BAL)[39] inside the BAL: a so-called internal oxygenator.

Do BALs support drainage of bile?

Another aspect of current BALS is the universal absence of functional biliary excretion into an isolated compartment. Liver cells in 3D configuration can form functional canaliculi, but it is unknown to what extent biliary compounds still accumulate intracellularly and whether these will shorten the vitality of the cells. If some export of biliary compounds occurs at the basal lateral side to the plasma compartment a hybrid system removing them from this compartment is a logic next step. This might mean a modular system in which a BAL is combined with an artificial liver support device such as hemodialysis, charcoal hemoperfusion or albumin dialysis[40].

Table 1 BALs to be commercialized

Company	Device	Characteristics	Clinical experience & future plans
HepaLife	Hepa-Mate™ (previously HepatAssist)	Cryopreserved porcine cells, treatment 3-6 h for 1-5 d, charcoal column, and centrifugal plasmapheresis. Cell mass previously 60 g, in future trial 160 g	Phase Ⅱ/Ⅲ with HepatAssist in 171 ALF patients, only 9% improvement in OLT/NR as compared to controls. New trial in preparation
Vital therapies	ELAD®	Two-chambered hollow fiber cartridge with immortalized human C3A cell line. Treatment up to 10 d. Ultrafiltrate perfusion. 4 replaceable cartridges. Cell mass 4 g × 200 g	Controlled study with 25 ALF patients completed. 92% recovery OLT/NR. Controlled clinical trial in 49 AoCLD patients in China
Beijing and Nanjing Universities	TECA-BALSS/HBAL	Porcine cells (10-20 billion cells), outside compartment of hollow fiber devices	Phase I, 15 patients ALF and 3 patients AoCLD
Hep-Art	AMC-BAL	Perfused scaffold, oxygenation *in situ*, 10-15 billion freshly isolated SPF porcine hepatocytes	Phase Ⅰ/Ⅱa; 14 ALF patients. Safe, no PERV transmission

BAL: Biological artificial liver; ALF: Acute liver failure; OLT: Orthotopic liver transplant; AoCLD: Acute on chronic liver disease; PERV: Porcine endogenous retrovirus; NR: Native recovery; SPF: Specified pathogen-free; ELAD®: Extracorporeal Liver Assist Device; BALSS: Bioartificial Liver Support Systems; HBAL: Hybrid Bioartificial Liver; AMC-BAL: Academic Medical Center University of Amsterdam-Bioartificial Liver.

How long do cells remain viable and functional?

Cell viability is of paramount importance for the life supporting capacity of a BAL. The experience is that primary liver cells in a bioreactor lose functionality over time. With this already being the case under optimal culture conditions, it is especially problematic when the environment of cells is 100% human plasma. A decrease in function is even more marked if cells have to live in the plasma of ALF patients[41-46]. Increased concentrations of toxic products and probably decreased concentrations of essential nutrients play a role in this regard. For this reason, BALs are only temporarily sufficiently functional and have probably to be replaced after a critical time by fresh ones.

Which cells can be used in the BAL?

Freshly isolated or cryopreserved porcine liver cells or a human hepatoma cell line have been most frequently used as the biocomponent in clinically applied bioartificial livers.

Because of the xenotransplantation-related disadvantages of porcine cells (immunological reactions and possible pig endogenous retrovirus transmission)[47-50] and the shortage of primary human hepatocytes, a well-differentiated human liver cell line seems to be the Holy Grail. Such a cell line will have minimal immunogenicity, no risk of xenozoonosis and required functionality and availability.

Primary sources for the development of such human cell lines are human liver tumor derived cell lines, immortalized fetal or adult hepatocytes and stem cells of hepatic, hematopoietic, mesenchymal or embryonic origin. However, in all cell types tested so far, the *in vitro* differentiation cannot be stimulated to such an extent that functionality reaches that of primary human hepatocytes. The future lies in having more insight into differentiation-promoting factors and the influence of matrix and co-culture conditions on the functionality of liver cell lines[51].

What is the current situation?

A few BAL systems are currently in the process of being commercialized (Table 1).

Vital Therapies just finished a controlled clinical trial in 49 AoCLD patients in China. At its website (www.Vitaltherapies.com) one can read: "The pivotal China trial enrolled 49 patients and was carried out to support the registration of ELAD in China. It demonstrated statistically significant improvement in transplant free survival for acute-on-chronic liver failure patients treated with ELAD compared to the control group. These were mostly hepatitis B patients. VTI filed an application for marketing approval with the China SFDA in September 2007 and this application remains under review. These results remain to be confirmed in studies outside China".

HepaLife (www.hepalife.com) is promoting the Demetriou system (formerly brought by Circe and Arbios) that is based on cryopreserved porcine liver cells combined with a charcoal column connected to a plasmapheresis circuit. More than 200 patients have been treated by this system. In a multicenter controlled clinical trial in 181 ALF patients, time to death was significantly prolonged only in a subgroup of 83 patients with ALF of known etiology.

The Chinese ALSDs (TECA BALSS and HBAL) and the Dutch AMC-BAL have been tested in Phase 1-2a trials but are not yet commercially available.

Why is clinical proof of efficacy rather limited?

There are a few explanations: (1) The hardware used for bioreactors has not always been optimal. Hollow fiber-based bioreactors will have mass transfer restrictions and the absence of an internal oxygenator will limit cell functionality if plasma perfusion is the approach to the patient's blood circulation. In addition not all BALs have a 3D configuration of the liver cells. (2) The optimal human liver cell line is still not available. Hepatoma-derived cell lines are not fully differentiated, nor are immortalized liver cell lines. Future developments in this regard are urgently needed. (3) In the already published clinical trials, patient populations have been rather diverse making intention-to-treat analyses disappointing. (4) If BAL treatment is applied in ALF patients to bridge the waiting time for OLT, post-transplantation survival is not only dependent on BAL treatment but also on OLT.

CONCLUSION

Taking all these considerations together there is certainly a future for the BAL, based on pre-clinical data and the lessons that have been drawn from the existing controlled trials. A well-differentiated human liver cell line is still the Holy Grail. If this cell line were available, future clinical trials should be done with it in a BAL consisting of minimal mass transfer restrictions and equipped with cell oxygenation *in situ*, loaded with a sufficient 3D mass. Eventually it should be combined with albumin dialysis and refreshed after a critical time. The trial population should be as homogeneous as possible and well defined with regard to survival capacity.

POSSIBLE CONFLICT OF INTEREST

Chamuleau RAFM is CSO of Hep-Art Medical Devices B.V. that produces the AMC-BAL.

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