Bioartificial liver support

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VG Bain, JL Montero, M de la Mata. Bioartificial liver support. Can J Gastroenterol 2001;15(5):313-318. Bioartificial liver support has been increasingly the focus of both basic and clinical research in an attempt to replicate the multiplicity of normal liver function. The concept is attractive because, if it is effective, patients with acute liver failure may be supported until native liver regeneration occurs or, by optimizing their condition, until liver transplantation is possible. Current bioartificial liver support systems utilize primary porcine hepatocytes or transformed human hepatocytes, which are housed within a bioreactor, through which the patient's blood or plasma is pumped in an extracorporeal circuit. The optimal source for the hepatocytes is an area of debate; however, a genetically engineered cell line may provide optimal function. Novel three-dimensional matrices that anchor the hepatocytes are being designed to mimic architectural features of the normal liver. Large multicentre, randomized, controlled trials are ongoing following several pilot studies. Serious side effects such as hemodynamic instability and immune reactions have been infrequent. Much controversy, however, surrounds the issue of possible transmission of pig endogenous retrovirus to humans, and current trials are being carefully monitored. Bioartificial liver support is a promising technology, and the results of current and planned studies are awaited with great interest.

Key Words: *Acute liver failure; Artificial liver support; Bioreactors; Hepatocyte culture; Xenotransplantation*

Foie bioartificiel

RÉSUMÉ : La mise au point d'un foie bioartificiel fait de plus en plus l'objet de recherche clinique et fondamentale en vue de reproduire les nombreuses fonctions du foie normal. Le concept suscite beaucoup d'intérêt parce que, si jamais la recherche porte fruit, les patients atteints d'insuffisance hépatique aiguë pourront être soumis à un traitement de soutien jusqu'à ce qu'il y ait régénération des cellules du foie ou jusqu'à ce que la greffe soit possible, en voyant leur état optimisé. Les méthodes actuelles de fonctionnement hépatique bioartificiel se composent essentiellement d'hépatocytes porcins ou d'hépatocytes humains transformés, placés dans un bioréacteur dans lequel passe le plasma ou le sang du patient par un circuit extracorporel. La source optimale d'hépatocytes fait l'objet de débats, mais une lignée cellulaire génétiquement modifiée peut assurer un fonctionnement optimal. De nouvelles membranes en trois dimensions permettant la fixation des hépatocytes ont été conçues de manière à reproduire l'architecture du foie normal. D'importants essais cliniques, multicentriques, avec groupe de contrôle et hasardisation sont en cours à la suite de plusieurs études pilotes. On a relevé peu d'effets indésirables graves comme l'instabilité hémodynamique et les réactions immunitaires. Il y a toutefois une controverse importante entourant la transmission possible du rétrovirus endogène du porc aux humains, et les essais actuels font l'objet d'une surveillance étroite. Le foie artificiel s'avère une technologie prometteuse, et les résultats des études actuelles et futures sont attendus avec grand intérêt.

There is no treatment for the most severe forms of acute and chronic liver failure except liver transplantation. Acute liver failure (ALF) is particularly devastating because of rapid progression to life-threatening complications such as cerebral edema, coagulopathy and multiorgan failure. This group of patients is a great challenge to liver transplant teams, with survival rates of only 50% to 60% compared with 80% to 90% for chronic liver failure (1). Furthermore, many patients with ALF die while awaiting liver transplantation (2). ALF has, therefore, been a logical first target for the development and application of bioartificial support. The hope has been that, in some cases (eg, acetaminophen hepatotoxicity), these systems could provide support while the native liver regenerates and thereby obviate the need for a transplant. In other cases, a liver support system would help to stabilize the patient and optimize his or her condition until a donor liver becomes available. Effective artificial liver support could ensure that ever-increasingly sparse

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TABLE 1
Artificial liver support systems

Artificial liver support	Bioartificial liver support
Plasma exchange	Extracorporeal liver perfusion (human or large animal)
Exchange transfusion	Cross-circulation (human or large animal)
Charcoal hemoperfusion	Bioreactor-based systems
Hemodiabsorption	Immobilized enzymes or liver cell microsomes

donor livers are used as efficiently as possible. The development of artificial liver support, however, has been a daunting task because the liver performs numerous essential functions, including gluconeogenesis, protein synthesis, amino acid metabolism, urea synthesis, lipid metabolism, drug biotransformation, toxin removal and bacterial clearance by Kupffer cells as part of the reticuloendothelial system. This complexity led to the concept that mammalian cells were needed to support liver function (3).

DEFINITIONS

- **Artificial liver support:** Nonbiological technologies (no cells or cellular components) based on the assumption that the patients' blood may be detoxified by removing small dialyzable toxins.
- **Bioartificial liver support:** Hepatocytes are used in some form to provide support to the failing liver. In addition to detoxification, a certain capability for metabolic function is provided, but this remains poorly defined.
- **Bioreactor:** Hepatocytes engrafted into a dialysis-like cartridge for use in a bioartificial liver support system.
- **Hybrid liver support:** Both biological and nonbiological components are used to provide liver support. This term is being used less frequently because most bioartificial systems are hybrid systems.

Examples of these systems are summarized in Table 1. These many different designs attest to the long appreciated need for liver support (4,5). The nonbiological support systems have been unable to improve outcome when tested in controlled trials, although such trials are generally lacking (6). Despite improvement of hepatic encephalopathy with artificial liver support systems, survival was not altered (7). This review focuses on bioartificial systems using bioreactors because these hold the greatest promise, and this is where most research activity is being concentrated (8-13). In addition, bioartificial livers are currently being tested in large multicentre trials.

HOW DOES A BIOARTIFICIAL LIVER WORK?

Whole blood or plasma from the patient is pumped through an extracorporeal circuit via a veno-venous access. The bioreactor becomes clotted unless some form of anticoagulant is administered, such as heparin or citrate, especially if whole blood is the perfusate. The circuit contains the bioreactor cartridge that houses the liver cells, which are supported by a variety of matrix types (Table 2). Provision of some form of matrix so that the hepatocytes can anchor appears to augment their differentiated functions (14). For example, the HepatAssist (Circe Biomedical Inc, USA) system uses collagen-coated dextran microcarriers, which improve viability (both before and after cryopreservation), cell transport and bioreactor seeding, cell polarity and func-

TABLE 2
Bioartificial liver support systems

System (reference)	Hepatocyte source	Estimated liver cell mass (g)	Bioreactor matrix	Other features	Used in humans
Hepatix* (8)	Immortalized human cell line "C3A"-hepatoblastoma derived	200	Hollow-fibre	Large hepatocyte mass perfusate is whole blood	Yes
HepatAssist† (9)	Primary porcine hepatocytes	70	Hollow-fibre, hepatocytes on collagen-coated microcarrier	Charcoal column plasma separator	Yes
Amsterdam Medical Center (10)	Primary porcine hepatocytes (Amsterdam)	‡	Nonwoven polyester	Primitive architecture Oxygenator integrated into bioreactor	No
Berlin (11,12)	Primary porcine hepatocytes	20	Woven capillary	Low diffusional gradients membrane, multifunctional capillaries	Yes
Minneapolis (13)	Primary porcine hepatocytes	50	Collagen gel in extracapillary space of hollow-fibre bioreactor	Three compartments bioreactor	No

**Vitagen Inc, USA; †Circe Biomedical Inc, USA; ‡Small bioreactor for investigative purposes*

tion (15). Newer bioreactors are moving away from the traditional hollow-fibre matrix design in an attempt to recreate a primitive hepatic architecture with better and more consistent cellular oxygenation, and low diffusional gradients to facilitate exchange of macromolecules between the plasma or blood and the hepatocytes (13). It remains unknown whether these theoretical advantages will translate into clinically important advances compared with the hollow-fibre matrix bioreactors, which have seen more clinical use. Additional techniques for hepatocyte immobilization have included the use of glass plates, hydrogels, cell encapsulation and others (16).

In most but not all bioreactors, the plasma or blood is separated from the hepatocytes by a semipermeable membrane. It is anticipated that this separation (depending on membrane pore size) could provide immunoisolation of the xenohepatocytes with a twofold benefit – reduced destruction of the hepatocytes by human xenoantibodies and reduced immune side effects because the various protein products released by the support hepatocytes are less likely to reach the patient (17). After the blood or plasma is exposed to the hepatocytes in the bioreactor, the filtrate is returned to the patient (Figure 1). Other components are included on the circuit, such as filters, oxygenators, heaters and, in some systems, charcoal columns. The rationale for the latter is to remove toxins from the blood before it comes into contact with the bioreactor and thereby preserve the function of its hepatocytes (eg, HepatAssist). A typical 'run' on the bioartificial liver is 6 to 8 h/day, but with some systems (eg, Hepatix, Vitagen Inc, USA), continuous support is possible. Both the Hepatix and HepatAssist systems have already demonstrated the potential for large-scale production and rapid availability of bioreactors at the medical centre where the treatment is to be performed. With the HepatAssist system, for example, cryopreserved porcine hepatoctyes are maintained at each treatment centre. When a patient with ALF is deemed to require bioartificial liver support, the cells are thawed and seeded together with the microcarrier into the extracapillary space of the bioreactor cartridge.

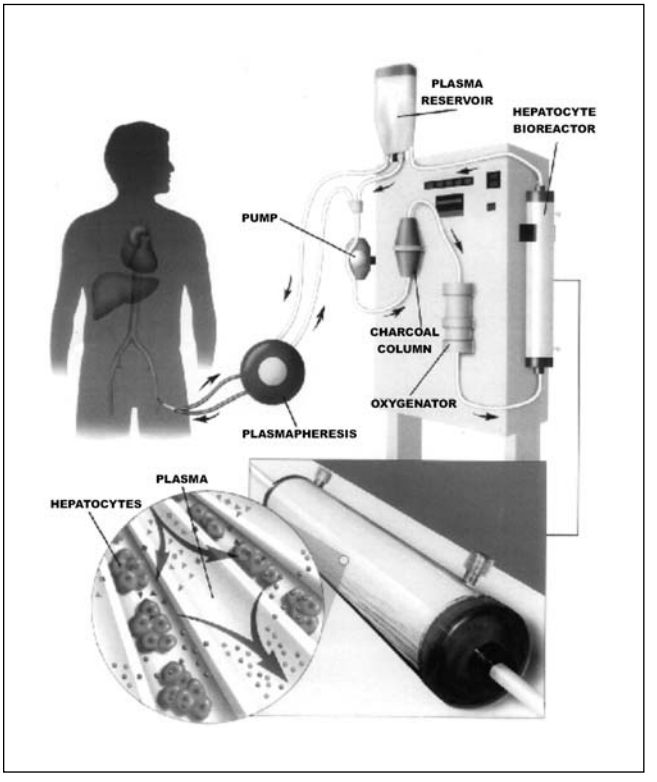

Figure 1) *The components of the HepatAssist system (Circe Biomedical Inc, USA) are shown in the upper panel. The lower panel shows the hollow-fibre bioreactor with hepatocytes within the fibres being perfused by plasma running between fibres (Figure courtesy of Circe Biomedical Inc, Lexington, Massachusetts, USA)*

EFFICACY IN ANIMALS

Numerous in vitro studies have tested a variety of different hepatocyte types as prototypes for bioartificial livers (16). These experiments have shown that cultured hepatocytes can maintain a variety of functions, including protein synthesis, gluconeogenesis, ureagenesis and biotransformation (phase 1 and 2). Studies in animal models of ALF followed. By using dog or pig hepatocytes in a hollow-fibre bioreactor to support dogs with ischemic liver failure, improvement was demonstrated in glucose control, ammonia and lactate clearance, and maintenance of blood pressure (9). By using acetaminophen in what had previously been shown to be a uniformly fatal model of ALF in dogs, survival of two of three dogs was reported using a bioartificial liver (8). Rabbits with galactosamine-induced ALF showed drug biotransformation function, histological evidence of hepatocyte regeneration, delayed onset of hepatic encephalopathy and prolonged survival compared with untreated controls when supported with a prototype hollow-fibre bioreactor (18). These and other animal experiments showed that a number of metabolic functions could be replaced and thereby provided the background to the initiation of human trials.

EFFICACY IN HUMANS

Occasional case reports have shown remarkable recoveries from a variety of liver insults (19), but this review is limited to the two largest experiences. The Cedars-Sinai group in Los Angeles, California, who have been leaders in this field, recently reported an update of their cumulative, uncontrolled experience, which included 31 patients, of whom 18 had ALF, three had primary nonfunction of a liver transplant (usually defined as failure of the transplanted liver within the first seven days after transplantation) and 10 had acute or chronic liver disease (20). Patients with hemodynamic instability or sepsis were excluded, and those with ALF had to be transplant candidates. Bioartificial liver support was provided with a hollow-fibre bioreactor containing 5×10^9 pig hepatocytes (less than 25 g of liver). It was perfused with patient plasma in an extracorporeal circuit, which also contained a charcoal column (HepatAssist [Table 2]). Of the 18 patients with ALF, 16 made a full

recovery after liver transplantation, one survived without transplantation and one died of pancreatitis. All three patients with primary nonfunction were successfully bridged to liver transplantation. Only two of 10 patients with acute or chronic liver failure were stabilized and successfully underwent transplantation; the remaining eight patients showed temporary improvement but ultimately died because they were not transplant candidates. Of interest, intracranial pressure (ICP) fell from a mean of 17 to 10 mmHg in the ALF group; however, only a marginal improvement in cerebral perfusion pressure was noted. The subgroup of patients with an initial high ICP (higher than 20 mmHg) had more substantial reductions after treatment as well as greater improvements in cerebral perfusion pressure. The ALF patients also demonstrated a significant improvement in the comprehensive level of consciousness score. Many biochemical parameters improved, but factor V levels and prothrombin times worsened, indicating that significant synthetic support could not be provided. Overall, this series demonstrated a 94% survival for ALF patients, which is unprecedented, but unfortunately the number of patients excluded is not reported, so the amount of selection that occurred is unknown. It is also impossible to know, in an uncontrolled trial, how much of the success should be assigned to the bioartificial liver and how much should be attributed to the transplant team, ie, how many of the 16 ALF patients would have survived if they had undergone transplantation without having received any bioartificial support.

The Kings College Liver Unit in London, United Kingdom has reported the largest experience using bioartificial liver support in ALF in a randomized, controlled trial (21). They enrolled 24 patients who were recruited into two separate groups. Group 1 (n=17) comprised patients believed to have a significant chance (30% to 50%) for spontaneous recovery with intensive care unit support only, while group 2 patients had already fulfilled criteria for liver transplantation. All patients were then randomly assigned to receive either bioartificial liver support (Hepatix) or standard therapy. Two Hepatix cartridges were used for a hepatocyte mass of 400 g. Survival was equivalent between patients receiving bioartificial liver support and those not receiving bioartificial liver support, both overall and within both subgroups. Seventy-eight per cent (seven of nine) of patients in group 1 receiving bioartificial support survived compared with 75% (six of eight) control subjects. Of group 2 patients, 33% (one of three) survived compared with 25% (one of four) without bioartificial support. An unexpectedly high survival in group 1 control subjects precluded any opportunity to show a survival advantage in Hepatix-treated patients. Ellis et al (21) pointed out that our prognostic estimates are imprecise, especially early in the course of ALF. Nevertheless, Hepatix-treated patients did show significant improvement in some aspects of liver function, particularly reductions in ICP and less deterioration in encephalopathy grade. This trial is of particular importance because it highlights some of the difficulties that can be expected in future trials – improving outcomes with standard supportive measures, difficulty in accruing large numbers of patients except in multicentre trials and our limited ability to determine prognosis accurately.

WHAT TYPE OF HEPATOCYTES ARE BEST?

Which type of hepatocytes are best for bioartificial liver support continues to be a heated debate among the proponents of the different systems. To date, primary pig hepatocytes and C3A cells have been used almost exclusively. Primary pig hepatocytes are obtained from a freshly slaughtered animal by collagenase digestion. The advantage is an unlimited supply of cells; however, the animal husbandry costs of maintaining germ-free pigs is considerable. There is also the risk of xenozoonosis, which is discussed further below.

C3A cells are derived from a hepatoblastoma cell line (8). They can be obtained in sufficient numbers in cell culture for large-scale bioartificial liver production yet are strongly contact-inhibited. They are well differentiated, and there has been no evidence that they are tumorigenic in humans, although this is at least a theoretical concern, particularly in patients who go on to undergo liver transplantation and receive immunosuppression.

Ongoing research is exploring new sources of cells, including immortalized hepatocytes of pig or human origin. These cells are nontumorigenic and are believed to have adequate function; however, they may be outperformed in certain functions by primary hepatocytes (22).

The number of hepatocytes required for optimal or even satisfactory function of a bioartificial liver is unknown. It has been suggested that poor clinical results may be due to an insufficient hepatocyte cell mass. Empirical estimates of 300 g or 3×10^{10} cells have been cited, which roughly equates to 20% of the normal liver mass, as the necessary amount of liver tissue required to sustain human life. Unfortunately, no one knows the correct amount. It will likely vary widely depending on the type of cell used, the quality of the cells and perhaps the type of matrix support. Furthermore, even an acutely failing liver maintains some viable cells that could contribute to some liver functions. On the other hand, a patient with a largely necrotic liver may have supernormal requirements for some liver functions.

SAFETY ISSUES

There are a large number of potential risks to patients who undergo treatment with bioartificial liver support systems (Table 3). Previous artificial support systems have been associated with problems related to incompatibility between blood and the extracorporeal circuit (23). This can lead to platelet activation, leukopenia, complement activation and other problems. These have not been major problems with current systems and are especially minimized in systems using plasma separation. Thrombocytopenia has been a common problem in ALF patients treated with various extracorporeal circuits. These issues were carefully studied in the Kings College controlled trial described above

(21). This trial found no overall adverse effect on platelets, except in one patient with pre-existing disseminated intravascular hemolysis. One patient developed tachypnea, tachycardia and fever, which resolved when the bioartificial liver was disconnected. Four patients had very high heparin requirements (suggesting activation of coagulation), which resolved using a prostacyclin infusion. These issues will require further study in future trials, but in general, the few patients treated so far have tolerated it well (20,21).

A recent report has characterized the xenoantibody response in eight patients with liver failure who were treated with a bioartificial liver containing pig hepatocytes (24). Patients receiving more than one run on the bioartificial liver developed rising titres of immunoglobulin G and immunoglobulin M antipig xenoantibodies that were directed against the pig alpha-galactosyl epitope. This epitope is present on all mammalian cells except those of humans, apes and Old World monkeys. It has been implicated as a prime target antigen in pig to human xenografts. Furthermore, these antibodies demonstrated cytotoxicity against pig aortic endothelial cells (24). Further study is required to determine whether exposure of these antibodies to the hepatocytes within the bioreactor will limit the usefulness of bioartificial livers in patients requiring prolonged treatment.

The biggest safety issue and the one that has stimulated the most debate is the subject of xenozoonosis, which is the cross-species transmission of animal infectious diseases to humans via a transplanted organ or, in the case of bioartificial livers, via blood exposed to the animal's hepatocytes. Bacterial and fungal pathogens could be eliminated in germ-free animal colonies, but viruses and especially retroviruses remain the major concern. Pigs have been the focus of the most discussion because they are favoured as donors in xenotransplantations and because they are used most commonly as the source of cells for the bioreactors of bioartificial liver support systems. Like humans, pigs harbour retroviral genes integrated into their genome, but these viruses are distinct from those of humans. Porcine endogenous retrovirus (PERV) mRNA has been detected in many different porcine cell types including the liver (25). PERVs are incorporated into the porcine germline, and the porcine genome is estimated to carry 10 to 40 copies. Accordingly, development of swine that are free of PERVs is very difficult. The important question, therefore, is, "can PERVs infect humans?" Under experimental conditions, PERV has been passed from cultured pig endothelial cells to a human cell line (25). However, follow-up of patients who previously received porcine pancreatic islet transplants in Sweden (26) and patients connected via an extracorporeal circuit to pig kidneys (27) has failed to detect PERV mRNA in sera or lymphocytes using pol- and env-specific primers, PERV reverse transcriptase using an ultrasensitive polymerase chain reaction assay followed by Southern blot hybridization or antibodies to PERV by Western blot.

There has been much controversy about the potential risks of PERVs compared with the importance of pressing onward with xenotransplantation and other uses of animal cells such as bioartificial liver support (28-30). The issue has been made larger than simply another opportunistic infection with the suggestion that PERVs alone, or after recombination with human endogenous retroviruses, could spread beyond the transplantation patient population to the community at large. The effect of this controversy on the testing of bioartificial liver devices has been variable. In general, clinical trials are continuing, but there have been at least temporary suspensions of activity in some countries. Compared with xenotransplantation of organs, bioartificial liver devices are likely to be of less risk for transmission of PERV. Indeed, the Cedars-Sinai group has performed a retrospective analysis of their patients treated with bioartificial liver support and found no evidence of PERV up to five years after the procedure (31). All current trials have strict guidelines for the prospective testing for PERV in all trial participants.

TABLE 3
Potential complications and risks of bioartificial support

Hemodynamic instability
Electrolyte imbalance
Hypocalcemia (if citrate used as anticoagulant)
Hypomagnesemia
Hypokalemia
Hemolysis, leukopenia, thrombocytopenia
Problems related to the use of porcine hepatocytes
Acute or chronic sensitization
Porcine endogenous retrovirus
Bleeding (if anticoagulation used)
Pulmonary complications
Acute pulmonary hypertension
Pulmonary edema
Change in response to some drugs

FUTURE RESEARCH

Although an effective bioartificial liver would have immediate clinical use, there is still much to be learned about what support is actually being given. With current systems, hepatocytes are being used as 'black boxes' to provide whatever is required. Basic research to dissect out which liver functions are most critical to replace or supplement will lead to improved systems. Genetically engineered cells may serve as these 'supercells' of the future (32).

Most current clinical trials are concentrating on ALF and primary nonfunction; however, potential expanded indications yet to be tested include alcoholic hepatitis and liver failure following large hepatic resections. In addition, a subset of patients with acute decompensation of chronic liver disease might benefit; however, the phase 1 study results from the Cedars-Sinai group were disappointing (20).

At this point, there are only limited clinical data, which are insufficient to permit conclusions about the efficacy of bioartificial liver support in ALF. Furthermore, it is unclear whether this technology might find its principal role in the support of failing livers while regeneration occurs, or rather to stabilize patients until transplantation ('bridging'). It is promising that the two largest trials both showed the potential to reduce cerebral edema, which is the most common cause of death in these patients (20,21). In the future, bioartificial liver support could optimize the patient's condition and permit a semielective xenotransplant and thereby improve results in the highest risk patients. For the present, we must devise ways to test bioartificial livers better, especially in view of improved general care of patients with ALF, which makes detection of efficacy more difficult. We need better prognostic indicators, particularly early in the course of ALF where application of bioartificial liver support seems most logical and at a time before a liver transplant is desperately needed.

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