Mortality from fulminant hepatic failure (FHF) is high (50%-80%), although survivors have absolutely normal liver function. The only treatment option that is curative is liver transplantation. However, because of shortage of cadaveric organ donors and/or delay in their availability, only 10% of FHF patients ultimately receive a transplant. This has led to development of artificial liver support systems with an idea to bridge the time to transplantation and/or recovery from FHF. Initial support systems were based on the principles of hemodialysis, hemofiltration, plasma-exchange, and hemoperfusion through adsorbent media (e.g., charcoal). However, lack of clinical efficacy, problems of bioincompatibility and fear of loss of circulating hepatocyte-regeneration factors led to the search for alternate strategies. With the successful long-term propagation and culturing of human and pig hepatocytes, and the development of adequate biocompatible microcarrier modules, it is now possible to be implanted peritoneally but are subject to early destruction because of inadequate vascularization and immune attack from the host. Thus the major thrust is now to develop bioreactors, e.g., Extracorporeal Liver Assist Device (ELAD), Bioartificial Liver (BAL), etc. These contain human or pig hepatocytes Implantd on hollow-fiber ultrafiltration cartridges. The patient's blood or plasma circulates through these bioreactors and after clearance of toxic compounds (via ultrafiltration and metabolism in hepatocytes) and addition of synthesized products, is returned to the patient. This article reviews the genesis, the pros and cons, and the clinical experience of BAL support for FHF. [Indian J Gastroenterol 2003;22(Suppl 2):569-574]

Key words: Bioartificial liver support systems, liver transplantation

Limitation is the short period of time available in FHF during which a suitable liver must be obtained. An artificial hepatic-support system that could serve as a bridge to transplantation, or ideally to spontaneous recovery, would be the answer to most of the above problems.

Over the last 30 years, a variety of supportive therapies for patients with FHF have been developed, but none has proved effective. In view of these artificial systems underestimated the complexity of liver cell functions. The concept of bioartificial liver support system (BALS) is a novel approach to circumvent this shortcoming and utilizes viable hepatocytes. The most popular mechanical device is a hollow-fiber dialysis cartridge containing living hepatocytes. These devices can be used with need for any immunosuppression.

Biological and non-biological liver support for acute liver failure

Earlier attempts to detoxify blood by using hemodialysis, hemoperfusion over charcoal and resins or immobilized enzymes, plasmapheresis and plasma exchange have not been very rewarding. The newer Molecular Adsorbent Recirculating System (MARS) enables albumin-bound toxins to be removed by dialysis, along with other dialyzable toxins, as it uses a double-sided, albumin-immunopurified polysulfone or hollow-fiber dialysis membrane or a molecular adsorbent in a close-loop dialysis circuit.

One of the primitive forms of biological liver support was extracorporeal liver perfusion, first reported by Eiseman et al in 1965. In general, arterial blood from the patient entered the hepatic artery and portal vein of an extracorporeal liver and exited from its inferior vena cava. There were no long-term survivors, but all patients showed some improvement in encephalopathy. The longest survival after the procedure was 8 days. A similar circuit using cadaveric human livers for extracorporeal perfusion was reported initially in 1966. It showed some success in the treatment of FHF before liver transplantation.

Hybrid liver support systems

Hybrid hepatic support combines the use of biological tissue with the use of non-biological materials. The use of hepatic tissue may provide synthetic, excretory and biotransformational functions, which combined with membranes removing cytokines and other toxins, is thought to be beneficial in FHF. Review of the early liver support techniques suggests that liver functions...
essential for survival would be provided best by mammalian liver preparations that allow sustained or repetitive application. These liver preparations are called BALS and contain biologic components within a synthetic framework. Biologic components may include isolated liver enzymes, cellular components, slices of liver, or cultured hepatocytes. Hepatocyte systems may be implanted in the patient or perfused extracorporeally.

Hepatocytes as cornerstone of BALS

Use of isolated hepatocytes has attracted a lot of attention. Development of techniques for preparation of single cell suspensions, and of cryopreservation of hepatocytes, indicate that these cells can be obtained as and when required. Isolated hepatocytes can either be used in an extracorporeal bioreactor or implanted within the patient in sites such as the peritoneum. Both these approaches are being explored experimentally. Surface area-to-volume ratio suggests that classic tissue culture technique of cell monolayers is clearly unsuitable for use in an extracorporeal system. It has now become possible to culture a large number of cells in a fairly small volume by the use of microcarriers, hollow-fiber support systems, multilayered moncultures separated by glass plates, or cell aggregates trapped between glass beads.9

Pros and cons of using 'bio' support

Hepatocyte systems can supply a greater number of liver functions in comparison with cell components or isolated enzyme systems. BAL can be constructed from semipermeable materials that provide a barrier between the hepatocytes and the host immune system. Therefore BAL therapy can be given without any immunosuppression, and hepatocytes from different species (xenocytes) can also be used within the BAL.12

Limitations of BAL systems include material and membrane interactions that are seen with other medical devices. For example, hepatocytes grown on a plastic surface with standard cell culture medium lose their gap junctions in about 12 to 24 hours, flatten and become agranular in 3 to 5 days, followed by death within 1 to 2 weeks. Improved techniques of cell culture have therefore become necessary for the application of BALS; these include addition of growth factors and hormones to culture medium, cultivation of cells in the presence of attachment factors and extracellular matrix constituents, co-culturing of hepatocytes with another cell type such as non-parenchymal liver cells or endothelial cells, cultivation of hepatocytes within a biologic gel or matrix, and use of transformed liver cells.14,15

Carriers for the hepatocyte

 Provision of some form of matrix for hepatocyte support seems to play an important role in maintaining differentiated hepatocyte function. Microcarriers, typically spheres of 100-300 μm diameter, of various materials, have been assessed for use in a BAL. Many microcarrier coatings optimized for use in tissue culture media are unsuitable for use in plasma, as they induce fibrin deposition, but materials such as Biosilon16 avoid this complication provided a minor level of anticoagulation is provided.16 Linear rates of carbohydrate metabolism, protein synthesis, and drug removal are achieved and sustained in plasma-perfused columns. Prolongation of survival and improvement of biochemical parameters of liver failure in rats with carbon tetrachloride- or galactosamine-induced liver failure have been reported.12 One potential advantage of the microcarrier-based system is the direct contact established between the blood or plasma and the hepatocytes, allowing free interchange of metabolites and synthesized proteins; this advantage is counterbalanced to some extent by the potential for cell debris, including DNA, to be carried into the body.

Hollow-fiber systems, in contrast, separate hepatocytes from blood which is pumped through the fibers.17 In a pilot study, reversal of paracetamol-induced hepatic failure was reported in two dogs.18 However, interposition of the membrane of hollow fiber is likely to retard significantly the diffusion of a number of larger molecules. Such devices are likely to help in the reversal of hepatic encephalopathy, which is probably due to accumulation of low-molecular-weight substances such as ammonia, mercaptans, fatty acids, phenols, and benzodiazepine-like molecules.17

Which hepatocytes for BALS?

The use of human cells is desirable but these can be obtained only from explanted livers in liver transplantation centers, or from hepatocyte specimens. Cultured fetal hepatocytes or immortalized lines from normal adult cells are potential prospects, but any manipulation that maintains hepatocytes as dividing cells gives rise to some disquiet, as does the use of tumor-derived cell lines. One recent pilot study reports the use of a well-differentiated human tumor-derived cell line which maintains the property of contact inhibition, so that cartridges of these cells can be maintained in a perfused system 'at the ready'.18

Xenocytes are another exciting possibility. It has been established that UV-B irradiation has a marked immunomodulatory effect. The survival and immunogenicity of UV-B irradiated hepatocytes in xenotransplantation using D-galactosamine acute liver injury model in New Zealand White strain suggest that UV-B irradiation alters the immunogenicity of hepatocytes probably by a T-cell independent mechanism. Advances in gene manipulation enable us to transfer genes.
Hepatocyte: which preparation to use for BAL?

Demetriou and colleagues were the first to demonstrate the feasibility of using microcarrier-attached hepatocytes for restoration of liver function. Microencapsulation in synthetic, spherical, polymeric structures composed of ultrathin membranes of cellular dimensions is another possibility. The membrane can be prepared from various polymers and its physical properties can consist of material or cells other biological active materials. The difference between the microcarrier is that this technique encapsulates the hepatocytes within a three-dimensional collagen matrix, whereas microcarriers carry the hepatocytes on its surface. A semi-permeable membrane separates the internal and external environment. The microencapsulated contents interact freely in biological reactions, while unwanted substances such as cells and antibodies cannot enter the microcapsules. This avoids the need for immunosuppression if transplanted. Microencapsulated hepatocytes have a limited functional viability of 4-6 weeks. Superior attachment surfaces such as Matrigel, a mouse sarcoma-derived complex protein that closely resembles liver basement membrane, and use of co-culture techniques have improved functional viability of isolated hepatocytes to 6-8 weeks.

Bioreactor designs

Three basic designs have emerged: bioreactors for use with suspension culture, bioreactors based on cell immobilization, and those with membranes. Most bioreactors have used capillary membranes within a cartridge for cell attachment. Capillary membranes allow a number of other functions to take place (gas exchange, substrate supply, and waste removal) efficiently and with practical ease. Hepatocytes may be seeded, cultured and grown within capillary membranes and perfused in the extracapillary space providing mechanical and physiological protection from toxic blood or plasma. This approach has been used in the three-compartment gel entrapment bioreactor. This entraps porcine hepatocytes in a collagen matrix incoated into the capillary lumen spaces of two 100 kDa molecular mass cut-off hollow-fiber bioreactors. An alternative approach is to construct a bioreactor with hepatocytes in the extra-capillary space with capillary membranes providing the inflow and outflow of media, oxygen, nutrients, toxins and waste. Capillary membrane constructions rely on transmembrane diffusion for mass transfer and so choice of materials is also of paramount importance. Construction of these artificial liver support devices that have had clinical exposure will be reviewed: Extra-capillary Liver Assist Device (ELAD), BAL, and hybrid liver support system.

ELAD, developed by Sussman et al. in 1992, incorporates the C3A cell line. This is a highly differentiated clonal population isolated from a human hepatoblastoma cell line, HepG2. Two hundred grams of cells were originally seeded and grown in the extravascular space of a hemodialysis cartridge containing >10,000 hollow fibers with a surface area of 2 m². Two to four weeks are required for adequate number of cells to have grown for clinical use. They have been shown to have good hepatocyte function after 8 months of storage. The intra-capillary space is used in the growth period for culture medium and oxygen supply and later in clinical use this space is used for perfusion of blood. The membrane has a molecular weight cut-off of 70,000 kDa, protecting hepatocytes from flow trauma, white cells and immunoglobulins, but allowing middle molecules and ammonia to pass. Recent modifications include two main design changes, i.e., increased cell numbers used per cartridge (700 g) and adaptation of the circuit so that it can be perfused with plasma and not blood.

The BAL system is conceptually similar to ELAD but originally had three major differences: the cell source, i.e., use of primary pig hepatocytes rather than a human tumor-derived cell line; the perfusate, which is plasma rather than blood; and the presence of a charcoal column filtering the plasma prior to its entry to the bioreactor. Hepatocytes are isolated from pigs and attached to collagen-coated dextran microcarriers. More recently, cryopreserved cells have been used. The hollow-fiber bioreactor consists of a polycarbonate cylinder containing cellulose nitrate/cellulose acetate porous fibers. Fibers have a pore size of 0.2 mm and a total internal surface area of about 6000 cm². The total extracapillary surface area is 7000 cm². The BAL system comprises a plasma separator, generating plasma (80-105 mL/min) from venous blood and passing this to the charcoal column. The plasma is then directed across the bioreactor at high flow rates (220-500 mL/min) which allows several passes before it is passed back to the individual.

Gerlach et al. described a more sophisticated hybrid liver support system. The structure is housed in a polyurethane PUR-725 case. The bioreactor is made up of several interwoven, independent polyurethane capillary systems, entering and leaving the bioreactor in four discrete bundles and each serving a different function. The four capillary bundles provide plasma in-flow, oxygen supply and carbon dioxide removal, plasma out-flow, and sinusoidal endothelial co-culture. Pig hepatocytes are seeded in the extra-capillary space and find all types of bundles locally, thus reducing transmembrane diffusion gradients. The design can be adapted by the addition of further bundles, allowing additional functions to be incorporated.

Hollow fiber specifications

Fibers with porosity less than 0.2 mm exclude cellular immune components, whereas fibers with a nominal
molecular cut-off below 100 kD exclude most complement proteins and immunoglobulins. Due to the importance of albumin in the transport of bilirubin and other waste molecules, a BAL membrane should be permeable to albumin (molecular weight: 65 to 70 kD). Hepatocyte functions are difficult to maintain at the high cell density, and new designs are being suggested to prolong hepatocyte functions. Many designs use hollow-fiber cartridges, which offer a large mass transfer area for volume, in order to enhance nutrient transport to the cultured cells. In addition to the engineering issues of mass transport and fluid mechanics, the BAL must fulfill certain biologic requirements in order to support a viable, functioning hepatocyte cell mass. For example, different membrane materials and immobilized biomolecules, such as lectins, may improve hepatocyte attachment and increase the detoxification activity of cultured hepatocytes.

**Clinical trials**

Non-biological and some biological methods of liver support have been tried extensively in *in vitro* and *in vivo* animal experiments, and in clinical trials (Tables 1 and 2). ELAD and BAL have also been subjected to animal and human clinical trials. Results, though preliminary, are very promising and suggest that these devices may prove to be useful bridges to transplantation, and in developing world, where liver transplantation is not available, as a bridge to spontaneous recovery.

The synthesis of foreign plasma proteins, coagulation factors and transport factors by the pig hepatocytes has significant immunological effects on the host, including antibody formation. Coagulation factors produced by the pig hepatocytes used in the BAL have been shown to cause immune complex deposition in animal and human studies and may contribute to end-organ dysfunction. Some investigators have avoided this problem by manipulating cell lines and abrogating protein production. The use of xenogeneic material also raises the possibility of the transfer of viral or prion disease to the host and is an area that is unclear and highly controversial.

**Future prospects**

The evolution of the BAL can be considered to be in infancy at present. Recent biotechnological developments in areas of cell-surface interactions and bioengineering have opened up many avenues in this field. Some of these designs have satisfied the rigorous of animal testing and are now being studied in phase I clinical trials. It is not clear which of them will emerge as an ideal BAL support device. A lot of work in standardization and quantitation of the source and number of hepatocytes and the mass of liver tissue used in such devices is still needed. Randomized clinical trials are necessary to establish the value of BAL therapy to patients with hepatic failure. Patients with FHF have a poor prognosis for survival and may benefit significantly from short-term BAL support. Although some authors recommend that controlled studies should be performed only in liver transplant centers, the situation in developing countries is vastly different and the facilities of liver transplantation are not available at all.

The ultimate test for BALs would be an improvement in

---

**Table 1: Non-biological hepatic support in man**

<table>
<thead>
<tr>
<th>Hepatic support</th>
<th>Subjects</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemodialysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keynes et al.</td>
<td>AHF, CHF</td>
<td>Improved neurological outcome, improved encephalopathy</td>
</tr>
<tr>
<td>Opolon et al.</td>
<td>9 patients</td>
<td>Decreased urea, creatinine, bilirubin</td>
</tr>
<tr>
<td>Denis et al.</td>
<td>41 patients</td>
<td>Decreased urea, creatinine, bilirubin</td>
</tr>
<tr>
<td>Klamt et al.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitzen et al.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hemofiltration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bellomo et al.</td>
<td>Animal and humans</td>
<td>Reduced IL-6, IL-1, TNFα, complement (C1q, C3a, C5a) levels, improved clinical status</td>
</tr>
<tr>
<td>Ronco, Bellomo</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Adsorbents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charcoal</td>
<td>AHF, CHF</td>
<td>Improved neurological status/survival</td>
</tr>
<tr>
<td>Gazzard et al.</td>
<td>22 patients</td>
<td>Improved neurological status/survival</td>
</tr>
<tr>
<td>O'Grady et al.</td>
<td>137 patients</td>
<td>No benefit in survival</td>
</tr>
<tr>
<td><strong>Hemodialysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash et al.</td>
<td>AHF, CHF</td>
<td>Decreased bilirubin, lactate, creatinine. No survival benefit</td>
</tr>
<tr>
<td><strong>Plasmapheresis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larsen et al.</td>
<td>12 patients (AHF)</td>
<td>Increased survival</td>
</tr>
<tr>
<td>Clemenon et al.</td>
<td>16 AHF, 11 CHF</td>
<td>Improved ICP, CBF, CPP</td>
</tr>
<tr>
<td>Larsen et al.</td>
<td>40 AHF, 11 CHF</td>
<td>CO, MAP</td>
</tr>
</tbody>
</table>

AHF: acute hepatic failure; CHF: chronic hepatic failure; IL: interleukin, TNF: tumor necrosis factor, ICP: intracranial pressure, CBF: cerebral blood flow, CPP: cerebral perfusion pressure, CMRO2: cerebral metabolic rate of oxygen, CO: cardiac output, MAP: mean arterial pressure
Table 3. Hybrid hepatic support in man / animals
Biotractor | Hybrid hepatic support | Subjects | Parameters | Outcome
--- | --- | --- | --- | ---
BAL (Uncontrolled trials) | Chen et al | Plasma-perfused cryopreserved pig hepatocytes (6x10^9) attached to dextran-coated microcarrier beads packed into extravascular space of hollow-fiber biotactor | Group 1: 12 patients (ALF); Group 2: 8 patients (CHF) | Improved ICP, CPP; improved NH, bilirubin and glucose | 12 patients to OLT; 6/8 died
Watanabe et al | | | Group 1: 18 patients (ALF); Group 2: 3 patients (primary graft non-function); Group 3: 10 patients (CHF) | Improved NH, bilirubin and glucose; Improved ICP, CPP | 16/18 to OLT; 3 patients to OLT;
Cuervas-Mons et al | Cryopreserved allogenic hepatocytes | Controlled trial in pigs, 4 hours daily | ICP significantly decreased in treated animals | 2 patients to OLT;
ELAD (Pilot controlled trial) | Ellis et al | Blood perfused 200 g of C3A hepatostatoma cells in attachment culture on outer Group 1: ELAD 9 patients, control 5 patients | 1 patient decreased ICP Cholesterol after first 8 h was no different between groups, as was NH_2 Factor V levels and arterial ketone body ratios | Survival
Group 1: 50% chance of survival on admission | | | | 8/9 patients (89%)
Group 2: meet criteria for OLT on admission | | | | 4/5 patients (80%)

ALF: acute liver failure, BAL: Bioartificial liver, CHF: chronic hepatic failure, ELAD: extracorporeal liver assist device, ICP: intracranial pressure, CPP: cerebral perfusion pressure, NH: ammonia, OLT: orthotopic liver transplantation

survival, which can only be assessed at a center where liver transplantation is not available.

Other areas of future interest are: the use of fetal hepatocytes, which has not been adequately studied. Fetal tissue offers significant advantages in that it may be easier to maintain and can be transplanted across the histocompatibility barrier. However, some ethical questions may have to be resolved before fetal tissue can be safely used. Lastly, several recent tissue engineering studies indicate that careful selection of hepatocyte attachment substrates and polymer supports may produce bioartificial matrices which may improve hepatocyte survival and function in BALs.

References
Anand

Bioartificial liver support for fulminant hepatic failure


Correspondence to: G7 Anand, VSM. E-mail: anilanand@saty.com

S74 Indian Journal of Gastroenterology 2003 Vol 22 (Supplement 2) December