Liver failure occurs either as acute liver failure (ALF) developing de novo or as acute decompensation of chronic liver disease (ACLF). The clinical manifestations are fairly similar characterised by multiorgan dysfunction characterised by encephalopathy (HE), hypotension, and renal failure (HRS). Unlike chronic decompensation of end-stage liver disease, ALF and ACLF are potentially reversible. A supportive therapy which can tide over the acute period of crisis (and act as a bridge to liver transplantation in cases of ALF) can be possibly life-saving. This essential premise of possible reversibility has lead to the attempts over the past 40 years to develop an extracorporeal liver support system. Essentially, two types of liver support systems are under development. (A) Bio-artificial devices, using hepatocytes is based upon the concept that they can perform the functions of the failing liver and (B) Artificial devices, of which the one currently being tried extensively is the Molecular Adsorbents Recirculating System (MARS), utilising the principles of albumin dialysis.

**BIOARTIFICIAL LIVER SUPPORT DEVICES**

This form of liver support system has two components, a bioreactor and hepatocytes. Bioreactor is device which houses the hepatocytes allowing free exchange of molecules between plasma/blood and hepatocytes across a membrane. The membrane allows movement of ‘toxins’ as well as transport proteins like albumin, while preventing passage of immunoglobulins, complements or viruses and cells. The hepatocytes use oxygen and nutrients and detoxify toxins from the plasma. The metabolites thus generated are passed back into the plasma. Theoretically, viable hepatocytes in these systems should therefore reproduce the synthetic, detoxifying as well as excretory functions of the liver. Clearly, the best type of cells to use would be human liver cells but the supply of such cells is limited and they are difficult to grow in culture. Two alternative approaches have been tried; (a) an approach is been to produce cells using genetic engineering techniques to provide them with both the ability to grow in culture while maintaining with the desired functional capabilities; (b) another approach is to use primary hepatocytes obtained from a pig. Thus, the C3A hepatocyte line, a sub-clone of the ubiquitous HepG2 hepatoblastoma cell line, has been used in one system (the Extracorporeal Liver Assist Device [ELAD] developed by Sussman and colleagues). However, while these cells survive and replicate adequately, they are functionally not very competent. Another immortalized human hepatocyte cell line under investigation is HHY41, which retains many liver-specific functions, protein synthesis, gluconeogenesis, and cytochrome P450 activity and is particularly resistant to acetaminophen. The escape of tumorigenic cells from the human hepatoblastoma cell line is a potential hazard in the
ELAD system. The addition of downstream cell filters to remove immortalized cells from the circulating fluid is generally regarded as being an adequate safety measure against seeding, but safety concerns remain. The second approach has been used in the HepatAssist device developed by Demetriou and colleagues, and the Academic Medical Center - BAL (AMC-BAL) developed by Chamuleau et al. The advantage of porcine hepatocytes, is that they can be satisfactorily cryopreserved, with cell isolation followed by storage at a clinical site prior to use, thereby avoiding the costs and contamination risks of long-term hepatocyte culture. However, as with human tumorigenic cells, safety concerns have been raised regarding the use of porcine cells too, specifically with respect to immune reactions to foreign antigens and xenozoonosis. Porcine endogenous retrovirus (PERV) is ubiquitous among bred pigs, and transmission to humans via BAL has been a persistent fear. PERV DNA and RNA have been detected in the supernatant of pig hepatocyte culture systems. In vitro studies have found that these viruses can infect human cell lines. However, PERV transmission to humans was not demonstrable in in vivo studies. A recent study also could not find any evidence of PERV infection, using reverse transcriptase polymerase chain reaction, 6 months after BAL treatment. In any case, these objections have lead to a moratorium against the use of such devices in the UK and most parts of Europe. The future of BAL clearly rests on finding the right hepatocyte.

Results of Clinical Studies using BAL. Data for the most important studies using the bioartificial liver support devices is summarised in Table 1. Similarly, none of the randomized trials evaluating the various BAL systems have demonstrated a clear survival advantage. The largest one showed a significant survival benefit only in the acetaminophen subgroup. Thus, while the clinical effects of liver support using hepatocytes have been tantalizingly interesting, a vast amount of work still needs to be carried out before any clear benefit is demonstrated beyond doubt.

EXTRACORPOREAL ALBUMIN DIALYSIS

The mechanisms underlying the development of the multi-organ dysfunction of liver failure are, as yet, poorly understood. The ‘toxin hypothesis’ implicates a variety of toxins which accumulate as a result of impaired hepatic metabolism/detoxification. Ammonia, protein breakdown products (aromatic amino acids, tryptophan, indole, mercaptan, phenol) and endogenous benzodiazepines, among others, are implicated in the development of hepatic encephalopathy. Nitric oxide (NO) and prostanoids are believed to be important in the pathogenesis of circulatory and renal dysfunction. Pro-inflammatory cytokines probably have wide-ranging influences, and oxidative stress has effects ranging from increased capillary permeability to modulating cell death. However the vast majority of these toxins (except possibly ammonia) are water-insoluble and albumin-bound, and conventional renal replacement therapy cannot effectively remove them.

Intravenous albumin administration is important in the treatment of patients with cirrhosis, and improves survival in those with spontaneous bacterial peritonitis or with hepatorenal syndrome, but the benefits exceed what could have been expected if it was acting simply as a volume expander. Some studies suggest that albumin is an important molecule involved in detoxification and binds various substances, and is perhaps more important in liver diseases than was previously thought. This is the basis for the use of albumin as a binding and scavenging molecule in devices based on albumin dialysis, and therefore the trial of such devices in patients with liver failure. There are three extracorporeal systems that are based upon the concept of albumin dialysis in clinical trials/application in patients and their current status will be briefly discussed below (Fig. 1).
The Molecular Adsorbents Recirculating System (MARS)
This device has been the most widely studied. In albumin dialysis, blood is dialysed against an albumin-containing solution across a suitable membrane. The albumin-bound toxins are potentially taken up by the binding sites of the dialysate albumin and thus removed from blood. Molecular Adsorbents Recirculating System (MARS) (Teraklin AG, Rostock, Germany) is based upon the principle of albumin dialysis and the system consist of three compartments - a blood circuit, an albumin circuit and a renal circuit (haemofiltration/haemodialysis). Blood flows through a hollow fibre dialysis module, where it is dialysed across an albumin-impregnated high-flux polysulfone dialysis membrane. 20% human albumin in the albumin circuit acts as the dialysate, and this is passed through the dialysate compartment of the blood dialysate, and is thus removed from the blood. The toxins are picked up by the albumin dialysate, which, in turn, is regenerated by haemofiltration/haemodialysis, followed by passage through two sequential adsorbent columns (containing activated charcoal and anion exchange resin), which remove most of the water-soluble and albumin-bound toxins and thus cleanse it. Substances with a molecular weight of more than 50 kDa such as essential hormones bound to carrier proteins, growth factors and albumin are not removed from the perfused plasma because of the pore size of the MARS membrane. Early in vitro studies showed effective removal of unconjugated bilirubin, drugs with a high protein-binding ratio (sulfobromophthalein, theophylline), and a protein-bound toxin (phenol) with MARS. In a recent study we have shown that toxins/drugs of a very wide range can be removed by albumin dialysis that has the potential to bind to albumin. We reported the efficient clearance of fentanyl, an opioid predominantly bound to a-1-acid glycoprotein.

Acute-on-chronic liver failure
After the initial studies showed that MARS reduced bilirubin and produced substantial reduction in the severity of HE and improvements in the circulatory and renal functions in patients with various forms and severity of liver dysfunction, the largest series of patients with ACLF (n=26), with intrahepatic cholestasis (bilirubin level > 20 mg/dL), treated with MARS was reported from Rostock. The series included 10 patients with a United Network Organ Sharing (UNOS) status 2b, all of whom survived, and 16 patients with a UNOS status 2a, of whom seven survived. Another study on patients with severe acute alcoholic hepatitis treated with MARS (n=8) showed improvement of 3-month predicted mortality (pre-MARS: 76%, post-MARS: 27%), with 50% of patients (4/8) still surviving at 3 months. The first randomized trial of MARS evaluated 13 ACLF patients with type-I hepatorenal syndrome who were treated with either MARS (n=8) or standard medical therapy including haemodiafiltration (n=5). The mortality rate was 100% in the group receiving haemodiafiltration at day 7 compared with 62.5% in the MARS group at day 7 and 75% at day 30, respectively (P < 0.01). Mean survival was longer in the MARS group, which was accompanied by a significant decrease in serum bilirubin and creatinine, and increase in serum sodium and prothrombin activity. MAP at the end of treatment was significantly greater in the MARS group. Although urine output did not increase significantly in the MARS group, four of the eight patients showed an increase compared with none of the control group.

The most recent (and largest completed) randomized controlled trial, performed in two centres (Rostock and Essen), included 24 patients with ACLF with marked hyperbilirubinemia (serum bilirubin >20mg/dL [340μmol/L]) who were randomized to receive standard medical therapy alone (n=12) or MARS in addition (n=12). The primary end-point of bilirubin <15 mg/dL for three consecutive days was reached in five of 12 MARS patients and in two of 12 control patients. Compared to controls, bilirubin, bile acids and creatinine decreased and MAP and HE improved in the MARS group. Most importantly, albumin

Table 1: Summary of controlled studies evaluating the bioartificial devices in acute liver failure (BAL and ELAD)

<table>
<thead>
<tr>
<th>Details</th>
<th>Study Demetrious et al. (2004)22</th>
<th>Ellis et al. (1996)23</th>
<th>Millis et al. (2001)34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient population</td>
<td>ALF (n=147), primary graft non-function (n=24)</td>
<td>ALF (n=24), Grp-I: not fulfilling LTx criteria (n=17)</td>
<td>ALF (n=24) 19 listed for LTx, five not listed</td>
</tr>
<tr>
<td>System used</td>
<td>Hepat-Assist (BAL)</td>
<td>ELAD</td>
<td>ELAD</td>
</tr>
<tr>
<td>Study design</td>
<td>Multi-center randomized controlled trial</td>
<td>Single center randomized controlled trial</td>
<td>Randomized controlled phase I trial</td>
</tr>
<tr>
<td>End-point</td>
<td>30-day mortality</td>
<td>In-hospital mortality or LTx</td>
<td>30-day mortality</td>
</tr>
<tr>
<td>Outcome</td>
<td>30-day survival: All patients: BAL 71%, controls 62%</td>
<td>Grp-I: ELAD 78%, controls 75%</td>
<td>Grp-II: ELAD 1/3, controls 1/4</td>
</tr>
<tr>
<td>Subgroups</td>
<td>All ALF: BAL 73%, controls 59% (P = 0.1)</td>
<td>Grp-II: ELAD 1/3, controls 1/4</td>
<td></td>
</tr>
<tr>
<td>Comments</td>
<td>Substantial impact of LTx (54% of all patients)</td>
<td>Survival among controls in Grp-I much higher than anticipated</td>
<td>Not adequately powered to look at outcome</td>
</tr>
</tbody>
</table>

ALF, BAL, bioartificial liver; ELAD, Extracorporeal Liver Assist Device.
Table 2: Summary of important studies evaluating the MARS device

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient population</th>
<th>Study design</th>
<th>End-point</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stange et al (2000)</td>
<td>ACLF with intrahepatic cholestasis (n=26)</td>
<td>Prospective case series</td>
<td>In-hospital mortality</td>
<td>UNOS 2a status: 7/16 survived</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UNOS 2b status: 10/10 survived</td>
</tr>
<tr>
<td>Mitzner et al (2000)</td>
<td>Type-I hepatorenal syndrome (n=13)</td>
<td>Randomized controlled</td>
<td>30-day mortality</td>
<td>Mortality: controls- 100% (day-7); MARS- 62.5% (day-7) and 75% (day-30) (p&lt;0.01)</td>
</tr>
<tr>
<td>Heemann et al (2002)</td>
<td>ACLF (n=24)</td>
<td>Randomized controlled</td>
<td>Primary: reduction of serum bilirubin</td>
<td>Improvement of bilirubin, and mortality 30-day with MARS (11/12 vs 6/11 controls, p&lt;0.05)</td>
</tr>
<tr>
<td>Jalan et al (2003)</td>
<td>ACLF due to acute alcoholic hepatitis (n=8)</td>
<td>Prospective case series</td>
<td>In-hospital mortality</td>
<td>Improvement of 3-month predicted mortality (pre-MARS: 76%, post-MARS: 27%), 3-month survival: 4/8</td>
</tr>
</tbody>
</table>

Acute liver failure

In the context of ALF, no controlled studies have been performed as yet, which is not surprising considering the difficult nature of this task. Novelli et al48 from Rome have treated nine cases of fulminating hepatic failure. Three patients survived without requiring transplantation. The remaining six were transplanted, of whom four survived, while two died due to sepsis. The authors requiring transplantation. The remaining six were transplanted, of whom nine survived. Haemodynamic and neurological improvements were noted following MARS therapy in most cases. Felldin et al (Gothenberg) describe 10 patients of ALF treated with MARS, of whom seven survived. Beneficial effect was most evident in those who received five or more sessions of treatment (4/5 survivors). A recent small randomised controlled study in patients with hyperacute liver failure found that a single session of MARS treatment (n=8) improved systemic haemodynamics (mean arterial pressure, systemic vascular resistance and cardiac output) compared to controls (n=5), who had only been mechanically cooled to match the MARS group.53

Prometheus

Another recently introduced system (1999) which has been presented as albumin dialysis, but which in reality utilizes somewhat different principles, is the fractionated plasma separation and adsorption (FPSA), based upon the principle of fractionation of the plasma with the subsequent detoxification of the native albumin by adsorption. It uses an albumin-permeable membrane with a cut-off of 250 kDa. Albumin, and possibly other plasma proteins with their bound toxins cross the membrane and pass through special adsorbers (one or two columns in series in the secondary circuit, containing a neutral resin adsorber and an anion exchanger) that remove the toxins. The cleansed albumin is returned to the plasma. The results of Prometheus treatment in 11 patients with ACLF and accompanying renal failure have recently been published.55 Improvement of serum levels of conjugated bilirubin, bile acids, ammonia, cholinesterase, creatinine, urea and blood pH occurred. A drop in blood pressure in two patients, and uncontrolled bleeding in one patient were the adverse events noted. Another study compared alternating treatments with MARS and Prometheus in five patients with ACLF. Reduction ratios of both bilirubin and urea were more with Prometheus. Their safety profiles were found to be comparable.56 Prospective controlled trials are planned for the future.

Single Pass Albumin Dialysis (SPAD)

The newly-developed SPAD system dialyses blood/ plasma against a 4.4% solution of albumin, which is disposed of after a single pass. A standard renal replacement therapy machine is used without any additional perfusion pump system, making the equipment required simpler. This fact, and the use of considerably more diluted albumin as the dialysate (4.4%, as opposed to 20% in case of MARS), offsets the cost of not recirculating the dialysate (in contrast to MARS). Continuous veno-venous haemodiafiltration can be undertaken in conjunction as well. In vitro studies suggest that its detoxifying capacity is similar to, or even greater than (especially with regard to bilirubin and ammonia clearance) that of MARS.57 However, clinical studies need to be performed before any conclusions can be drawn.

Conclusions

Mortality in patients with liver failure remains unacceptably high. Acute decompensation of cirrhosis carries as poor a prognosis as ALF and improvement in the outcome of these patients may be improved with earlier referral and emerging therapies. The bioartificial liver systems have failed to live up to their initial promise and currently cannot be recommended for the treatment of patients outside of carefully controlled clinical trials. Strategies using albumin dialysis are currently the only viable liver support therapy and have been shown to alter some of the pathophysiological mechanisms thought to be...
important in the development of liver failure but progressive thrombocytopenia, coagulopathy and uncontrolled sepsis are relative contraindications to the use of MARS. Results of large on-going trials are necessary to define the exact place of these emerging technologies in the management of liver failure.

REFERENCES


