Novel strategies for liver support in acute liver failure

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At present, the treatment of a patient in acute liver failure is based upon scrupulous intensive care. In those patients whose condition deteriorates, emergency liver transplantation must be considered. There would be great benefit if it were possible to provide treatment which would stabilise the condition of a patient in acute liver failure. Thus, there is great incentive to develop a means of artificial liver support. Over many years, a considerable array of therapeutic strategies has been investigated. These can be considered in four main categories: plasma exchange, haemofiltration, extra-corporeal liver assist devices (ELAD), extra-corporeal liver perfusion (ECLP). Finally, the role of xenotransplantation is considered.

Acute liver failure is defined as the development of liver failure within 8 weeks of the onset of jaundice. It is a complex syndrome resulting from the loss of synthetic and metabolic functions of the liver. Several aetiologies are associated with acute liver failure and demonstrate a marked geographical variability. In the UK, the most common cause of acute liver failure is paracetamol poisoning, whilst in the USA patients with viral hepatitis constitute the largest group. The aetiology has an important effect on the prognosis of an individual in acute liver failure; patients with paracetamol toxicity have a relatively better prognosis than those with non-A-non B hepatitis or drug induced hepatitis.

Symptoms and complications

Patients in acute liver failure develop encephalopathy which varies from mild impairment of cognitive function and lethargy to coma. The exact cause of the encephalopathy is unclear; many theories have been advanced including the production of false neurotransmitters, alteration in the ratio of straight chain to aromatic amino acids and elevated ammonia levels. The fact that there is no consensus indicates that the true cause is unknown. A frequent association with encephalopathy is the development of raised intra-cranial pressure and the consequent reduction in cerebral perfusion pressure. It has been suggested that the
rise in intra-cranial pressure is related either to a structural change in the blood brain barrier or osmotic effects resulting in astrocyte swelling\textsuperscript{11-14}.

Acute liver failure is often complicated by acute renal failure which has a major effect on prognosis. Renal failure, together with the loss of liver function, results in major imbalances in the acid base balance; an initial alkalosis develops into a profound acidosis\textsuperscript{15}.

With the loss of synthetic activity of the liver, a deficiency develops of a number of the components of the clotting cascade. The resulting severe coagulopathy is not only life-threatening in itself, but also complicates any medical intervention and invasive monitoring. Disseminated intra-vascular coagulation is a frequent associated complication\textsuperscript{16}.

Sepsis, particularly from chest infections, is a very serious risk. Patients in acute liver failure become immunosuppressed, possibly, in part, due to defective synthesis of hepatic proteins and complement\textsuperscript{17-21}. Indeed, it is often the development of raised intra-cranial pressure and sepsis that results in the death of patients in acute liver failure.

**Classical treatment**

At present, the treatment of a patient in acute liver failure is based upon scrupulous intensive care. Careful measurement of cardiovascular status using invasive cardiovascular monitoring allows optimisation of fluid and inotrope requirements in a patient who is likely to have very low systemic vascular resistance and concomitant sepsis. In the face of progressively deteriorating encephalopathy, positive pressure ventilation is often necessary. In those patients who develop renal failure, support by haemofiltration or dialysis is necessary\textsuperscript{22}.

Intra-cranial pressure is assessed clinically or more directly either by subdural or monitoring of transcranial Doppler studies\textsuperscript{23}. The importance of this lies in the associated reduction in cerebral perfusion pressure and the risk of irreversible cerebral damage. It is important to avoid procedures known to cause elevations in intra-cranial pressure (e.g. tracheal suction) and the patient is nursed in a head up position. Mannitol is used to reduce intra-cranial pressure and hyperventilation is another means to reduce the intra-cranial pressure\textsuperscript{22-24}.

N-acetyl cysteine is widely used in acute liver failure, particularly in cases of paracetamol poisoning; there is evidence of enhanced tissue oxygen utilisation\textsuperscript{25-27}. A number of other medical therapies have been used including the use of systemic steroids and the benzodiazepine antagonist flumazenil, but none have found a long standing role.
Stabilisation of a patient in acute liver failure

In those patients whose condition deteriorates despite the measures described above, emergency liver transplantation must be considered. In order to enable the decision to proceed to transplantation to be made in as timely a manner as possible, various criteria are used\(^3\)\(^,\)\(^28\). These are based on analysis of large series of patients in acute liver failure and, essentially, define the point at which the probability of spontaneous recovery falls below a certain level. Several problems are associated with liver transplantation for acute liver failure; by the stage that it is clear that the patient will not recover spontaneously; the life expectancy is likely to be no more than a few days. For this reason many patients die awaiting a suitable donor and those patients who do undergo transplantation are usually in a very unstable condition; the results of transplantation under these circumstances are, predictably, substantially worse than those where the patients are in a stable condition\(^1\).

For the reasons described above, there would be great benefit if it were possible to provide treatment which would stabilise the condition of a patient in acute liver failure. A patient who had fulfilled the criteria for emergency liver transplantation would be maintained in a stable condition until a suitable donor were available; fewer patients would die awaiting transplantation and the prognosis of those transplanted would be improved. In addition, provision of effective liver support would enable a patient to be maintained for longer whilst awaiting spontaneous recovery of liver function; indeed there is evidence of regeneration in many of the explanted livers from patients transplanted for acute paracetamol toxicity. It is possible, therefore, that some patients who would currently require transplantation might recover without a transplant.

For this reason there is a great incentive to develop a means of artificial liver support. Over many years, a considerable array of therapeutic strategies has been investigated. These can be considered in four main categories: plasma exchange, haemofiltration, extra-corporeal liver assist devices (ELAD), extra-corporeal liver perfusion (ECLP).

_plasma exchange_

The aim of plasma exchange is to replace the plasma of the patient with pooled donor fresh frozen plasma which includes those proteins normally synthesised by the liver (e.g. components of the clotting cascade). This also has the effect of removing the toxic elements within the blood which the damaged liver fails to metabolise. However, there is
little evidence that plasma exchange has any beneficial effect in respect of neurological function or reduction of intra-cranial pressure\textsuperscript{29,30}. It is used by many centres immediately prior to liver transplantation in an attempt to improve the condition of the patient. Although the replacement of coagulation factors and complement molecules is theoretically helpful, the rate of turnover of these molecules is such that, to maintain treatment for any length of time would place a huge burden on the provision of blood products.

**Haemofiltration**

Haemoperfusion is the process whereby the blood or plasma of the patient is passed through columns of activated charcoal. It relies on the ability of activated charcoal to absorb many of the middle-sized molecules which have been implicated in the development of the neurological complications of acute liver failure. Many of the clinical studies were performed during the late 1970s and 1980s\textsuperscript{31}. Initial problems occurred with bio-incompatibility and in particular problems of clotting, loss of platelets and the development of disseminated intravascular coagulation. Many of these problems were avoided by using columns that were coated to avoid the direct contact of plasma with the charcoal particles and also by using prostacyclin. As a result of these improvements, a very large number of patients in acute liver failure were treated using this device. Initial studies were very encouraging with suggestions of dramatic improvements. However, when randomised controlled clinical trials were performed, it was found that the use of charcoal haemoperfusion conferred no demonstrable advantage\textsuperscript{32}. This underlines the problems of analysing the effectiveness of treatment modalities in acute liver failure which represents such a wide range of pathological conditions; the outcome for a particular individual is extremely difficult to predict. For this reason, it is particularly important any new treatment modality is be performed using a randomised control protocol.

**Extra-corporeal liver assist devices (ELAD)**

There are already at least two commercially available 'bioartificial livers' in clinical use. The aim of the system is to produce a device in which a cartridge or 'bioreactor' is seeded with functional hepatocytes, which are then exposed to the blood or serum of the patient. By using a cartridge system seeded with cultured hepatocytes, it is possible to have a stock of
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primed cartridges allowing a readily available means of artificial liver support. The source of hepatocytes and the ability to maintain their viability have been two of the major technical issues facing this strategy. In one design, the hepatocytes are derived from an immortalised hepatoma cell line\textsuperscript{33}. These cells exhibit the characteristic of contact inhibition and will grow to form a mono-layer. The hepatocytes are seeded into capillary tubes which are, in turn, placed within the reactor chamber. After some successful initial results in an experimental model of acute liver failure, clinical trials were performed; a number of patients were improved both biochemically and haemodynamically, allowing 'bridging' to transplantation and even complete recovery in some patients. However, a more recent study failed to confirm these very positive results\textsuperscript{34,35}.

In another design, hepatocytes from pig livers have been used despite the theoretical problems of xeno-rejection\textsuperscript{36-37}. It has been shown that functional hepatocytes can be grown within the device and that functional capacity is recovered after freezing and thawing. These devices are restricted by the limited hepatocyte mass; current devices process only 120 ml blood/min as compared to the normal blood flow in an intact liver of 1.5–2.0 l/min. In addition, although the majority of hepatic parenchymal mass is composed of hepatocytes, other cell types are probably of great importance in the function of the organ. The microscopic structure is important in the excretory functions of the liver.

**Extra-corporeal liver perfusion (ECLP)**

A number of research groups are studying the potential use of whole livers for extracorporeal perfusion and liver support. This is not a new concept but, with recent improvements in cardiopulmonary bypass technology and developments in genetic engineering, interest in this as a possible therapeutic modality has increased.

Over the past 40 years, more than 100 patients in liver failure have been treated by extracorporeal liver perfusion using organs from a wide variety of sources\textsuperscript{38}. The fact that adequate liver function can be provided with an extracorporeal perfused liver has been recently demonstrated\textsuperscript{39}, using human livers which, for functional or logistical reasons, were not required for transplantation. In three patients with liver failure, effective liver support was demonstrated using a relatively simple perfusion apparatus. However, because of the world-wide shortage of organ donors, it is impractical to use human donor livers.
for extra-corporeal support. For this reason, attention has been directed to the use of organs from other species (xenoperfusion).

The transplantation of organs from one species to another is termed xenotransplantation. Xenografts were classified in 1970 as ‘concordant’ and ‘discordant’ according to the occurrence of hyperacute rejection, a rapid and catastrophic reaction within minutes or hours resulting in the loss of the graft; this correlates with the presence or absence of pre-formed anti-species xeno-antibodies. Hyperacute rejection is due to binding of xeno-antibodies and activation of the complement cascade resulting in endothelial cell damage and lysis. Concordant xenografts (e.g. chimpanzee to man) are rejected over a period of days in a manner analogous to allograft rejection by a previously unsensitised recipient (first set response).

Clearly there are major advantages in using livers from concordant species for clinical xenoperfusions. Baboons are concordant with humans and there have been many examples of ex vivo perfusions, cross circulations and orthotopic transplants between baboon and man. However, there are many problems associated with the use of primates in clinical transplantation; these include the size discrepancy between most primates and humans, serious concerns about primate-borne disease (zoonosis) and ethical/cultural objections to the use of primates.

For these reasons, many investigators have looked at the possibility of reducing the immunogenicity of discordant species pairings, by either altering the expression of sugar residues (the target antigen of xeno-antibodies) or by manipulating the process of human complement activation. In one such development, a strain of pigs has been genetically engineered to express human decay accelerating factor (hDAF), a down-regulator of human complement activation. There is good evidence that this confers some degree of protection from hyperacute rejection in models of transplantation. More recently, the use of livers from hDAF transgenic pigs for ex vivo liver perfusion has been investigated.

Whilst the immunological problems associated with xenoperfusions are well recognised, there are many technical issues associated with ex vivo perfusion of any organ outside its normal milieu. In an extra-corporeal circuit, all neural and endocrine homeostatic mechanisms have been abolished. The appropriate conditions required to keep an organ in optimal physiological condition are as yet poorly defined. Carrel and Lindbergh working in the 1940s and 1950s were able to maintain relatively normal organ function for several months in culture conditions, but only by perfusing with fresh serum from a vast number of animals.

The optimal design of a perfusion circuit remains a matter of debate. It is well established that perfusion of the liver with blood from the portal
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System is essential for normal liver function \textit{in vivo} but this would be difficult to achieve in an extra-corporeal circuit. Some investigators perfuse via both the hepatic artery and the portal vein\textsuperscript{47}, whilst others use only portal perfusion\textsuperscript{48}. Some use pulsatile flow to the arterial system, whilst others do not. A further consideration is that of the effect of respiration-induced pressure changes on hepatic venous drainage\textsuperscript{47}.

The role of adjuvant pharmacological manipulation in order to enhance liver perfusion and function remains unclear with little consensus between authors over the usefulness or otherwise of drugs such as prostacycline, insulin, superoxide dismutase or N-acetyl cysteine\textsuperscript{49}.

Development of this technology is further complicated by the absence of an objective and accurate means of assessing liver function. The clinically available liver function tests (bilirubin, transaminase, alkaline phosphatase) are more indicators of liver damage rather than liver function. A number of measures of hepatic metabolic activity have been tested in this context; these include metabolism of lignocaine (MEGX), galactose, theophylline. Alternatively, the synthetic activity of the liver can be measured using such parameters as Factor V, albumin, coagulation activity and complement.

Although there are little data regarding the physiological compatibility between man and the pig, the clinical studies carried out to date would suggest that porcine synthetic and metabolic activity are adequate, at least to provide partial and temporary liver function.

The history of clinical extra-corporeal liver perfusion dates from 1965\textsuperscript{50,51}, when the use of porcine liver xenoperfusions was described in patients with acute liver failure. Perfusions were only able to be maintained for up to 6 h and, during this time, there was some evidence of metabolic and synthetic function with improved neurological status. This early work was carried on by a number of investigators\textsuperscript{52-63}. Clinical perfusions were carried out using organs from a variety of species including pig, baboon, cow and human\textsuperscript{56}. It was noted that whilst perfusions using pigs livers could only be maintained for 6–9 h, baboon liver perfusions could be maintained for up to 24 h. If a human organ was used, perfusion could be continued for up to 60 h. Evidence of liver function was demonstrated with all species used although it was concluded that baboon xenoperfusion was more effective.

Histological studies\textsuperscript{56} of the perfused pig livers revealed evidence of ‘acute humoral xenograft rejection’, which was not seen if a baboon liver was used. A marked rise in the titre of species specific antibodies following exposure to pig livers was also noted and, indeed, one episode of anaphylaxis in response to repeated porcine xenoperfusions. Similar results were reported by other investigators\textsuperscript{57,58}. In view of the relatively poor clinical results obtained with extracorporeal liver perfusion and the
advent of other treatments including charcoal haemoperfusion and
haemodialysis, this technique fell into abeyance between the end of the
1970s and the early 1990s.

Current interest in extra-corporeal liver perfusion comprises a small
number of groups who have developed more rigorous pre-clinical
models. Advances in the technology of oxygenation, pumping and
temperature maintenance have reduced the technical complications of
the circuit. In addition to developing the circuit design, a number of
investigators have studied in the laboratory the effects of porcine
xenoperfusion with human blood.

A number of recent clinical trials have also been performed. Fox et al. have used both human and porcine livers in an extra-corporeal device in
patients with liver failure for periods of up to 3 days (EASL). This
duration of perfusion is in marked contrast with the experience of other
investigators. The donor pigs were not genetically modified; it is likely,
therefore, that this prolongation of perfusion is the result of improved
bypass technology. There was evidence of metabolic function through-
out although evidence of neurological improvement was more difficult
to confirm.

In another recent study, however, perfusion of a patient in acute liver
failure with a liver from a normal pig was possible for only for 3.5 h. Improved biochemical and neurological parameters were noted but the
patient developed vasodilatation and disseminated intra-vascular co-
agulation. The organ showed evidence of profound endothelial damage.
Other investigators used porcine livers for the treatment of 4 patients
in acute liver failure and were able to perfuse the organs for 2–5 h.
During this time, each liver perfusion resulted in an improvement in
coma grade and decrease in serum bilirubin and ammonia (one patient
underwent sequential perfusion with 5 livers). It is of note that, in spite
of initially normal levels of anti-species antibody, the perfused organs
failed to exhibit the degree of IgM or IgG staining anticipated and did
not show histological evidence of classical hyperacute rejection.
Similarly, there was no evidence of deposition of membrane attack
complex. It was suggested that the liver is less susceptible to hyperacute
rejection than the heart or kidney, an observation that is certainly
recognised in the clinical practice of liver transplantation. There was,
however, a significant increase in the titre of anti-species antibodies
following xenoperfusions.

All the recent clinical trials have utilised livers from genetically
unmodified pigs. It is difficult to assess to what extent the limitation in
perfusion is a function of the immunological disparity between the
species. Certainly there is evidence that the classical hyperacute
response is not seen; this may be related to the fact that patients in
acute liver failure have reduced circulating levels of complement. For this
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reason it is not possible to predict the impact of using transgenic organs, expressing genes that inhibit human complement activation. If the expression complement regulating genes in porcine livers enables prolongation of liver perfusion and function from 6 h to the 60 h (as seen using human livers), it is likely that extra-corporeal liver perfusion will provide therapeutically useful hepatic support.

In our laboratory, successful perfusion of pig livers with pig blood has been achieved for periods of at least 72 h. Investigations are in progress to assess the effectiveness of the hDAF transgene in prolonging the effective duration of perfusion of a porcine liver with human blood. These studies are a necessary precursor to clinical trials.

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