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The Use of Ex Vivo Xenogeneic Whole Liver Perfusion as a Bridge to Liver Regeneration or Liver Transplantation

George M. Abouna

There is a well-recognized need for an extracorporeal system that is practical, successful and cost effective for the support of patients with fulminant hepatic failure (FHF). In the USA alone, some 30,000 patients die each year from FHF, including some 1,400 or 10% of the patients who are on the liver transplantation waiting list.¹ FHF is usually a terminal event of severe liver dysfunction with resultant encephalopathy, accumulation of toxic metabolites, failure of synthesis of vital substances, failure in neurotransmission, renal failure, increased intracranial pressure, cerebral edema and death in 80-90% of patients. Clearly, therefore, the solution to this major and increasing problem is to develop an effective extracorporeal system that is capable of replacing all the functions of the liver, including detoxifying, excretory and synthetic, for a long enough period until the patient's own liver fully regenerates or until a compatible cadaver liver can be found for transplantation. It is logical that such a system will be one that utilizes a whole liver taken from a healthy animal, which is placed in a perfusion circuitry that recreates the normal physiological conditions of the *in vivo* liver. The animal liver will be connected to the patient after removal of the latter's preformed xenoantibodies in order to prevent hyperacute rejection of the perfused liver.

In the early 1960s-70s, several extracorporeal techniques were tried for the treatment of patients with FHF, including hemodialysis, exchange transfusion, hemoperfusion through charcoal or resin columns, and hemoperfusion through an isolated animal liver. It soon became clear that, of these techniques, extracorporeal xenogeneic whole liver perfusion was the most effective in reversing deep hepatic encephalopathy as it performed all of the

functions of a normal liver, thus giving the patient's failing liver sufficient time to regenerate and resume normal function.²⁻⁸ Unfortunately, with the subsequent development and improved success of liver transplantation in the 1980s, many patients with FHF were treated by emergency hepatic transplantation, and the procedure of extracorporeal liver perfusion was abandoned.^{9,10} While this did improve the survival rate of patients with FHF, treatment of this condition by liver transplantation is clearly complicated, expensive, and deprives other more suitable patients from receiving a liver graft. Also, liver transplantation may not always be indicated since many patients with FHF would recover spontaneously if they could be supported for some time by the extracorporeal liver device until their own liver regenerates, thus avoiding the need for hepatectomy, a complicated liver transplantation operation and lifelong immunosuppression.

Today, the realization has once again emerged of resuming extracorporeal hepatic support as the most logical method of supporting the ever-increasing number of patients with FHF and those awaiting liver transplantation. Unfortunately, because of failure to adequately appreciate some of the fundamental criteria which must be implemented if a successful extracorporeal liver is to function adequately, which were outlined in the 1970s,^{7,8} some centers have taken "short cuts", such as perfusing the liver through the portal vein only with inadequate maintenance of physiological inflow pressures and flow rates, and have thus failed to obtain the required successful outcome.¹¹ This again led to temporarily abandoning *ex vivo* liver perfusion.

Instead, there have been recent attempts to use bioartificial devices containing some 100-250

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grams of encapsulated porcine hepatocytes in hollow fiber columns,¹² to transplant hepatocytes obtained from human livers,¹³ or to carry out allogeneic or xenogeneic auxiliary whole liver transplantation.¹⁴ Bioartificial devices have not shown consistent and successful outcome in bringing patients out of deep coma for extended periods of time, largely because of the low viability and metabolic capacity of the very small number of cultured and cryopreserved hepatocytes in the artificial liver device. The transplantation of hepatocytes obtained from human livers has also been quite unpredictable, largely because of the unknown mass of hepatocytes required to carry out the functions of the normal liver. Also, the problem of scarcity of suitable human livers and of using a human liver in this way, instead of as an orthotopic liver graft, have provided an ethical dilemma. Likewise, auxiliary liver transplantation is a major and costly procedure, and again raises the ethical dilemma of using a human liver for temporary support instead of as a permanent graft. If a xenograft is used in this way, there remains the problem of hyperacute rejection and the negative consequences of immunosuppression.

Historical Perspective of Ex Vivo Liver Perfusion

The first use of an ex vivo xenogeneic liver perfusion system for the treatment of hepatic failure was reported by Eiseman, but unfortunately this failed to improve patient survival largely because of the non-physiologic conditions of the perfusion circuitry used and the unknown significance of preformed xeno-antibody and complement activation in the rejection of the ex vivo liver.² In the early 1970s, we devised the perfusion system which recreated the physiological conditions of a normal liver, and at the same time employed plasmapheresis before perfusion of the porcine liver in order to reduce the concentration of xenoantibody. When pig livers were initially used and when anti-porcine antibody returned and precluded use of another pig liver the liver of another animal species, e.g., a primate, was employed.^{3,4,6,15} Using these guidelines, 33 clinical ex vivo liver perfusions were carried out for the treatment of 21 episodes of Grade IV hepatic coma in 10 patients with acute viral or toxic hepatitis, decompensated chronic liver disease, or ischemic necrosis after failed liver transplantation.^{7,15} During this experience several important parameters were analyzed,

including the rate of clinical recovery in relation to the length of perfusion, the type of donor animal species used, the number of perfusions required to reverse coma, the incidence and type of xenograft rejection, the immunological changes in the recipient, and possible methods of removing the preformed antibodies prior to perfusion.

Extracorporeal xenogeneic liver perfusion consistently and repeatedly reversed deep encephalopathy when all other measures had failed. One patient was brought out of Grade IV hepatic coma on eight separate occasions over a period of 76 days while awaiting liver transplantation,⁵ while another three patients with acute viral hepatitis recovered completely and were discharged from the hospital. Two of these patients are alive and well today after nearly 28 years.^{6,15} It was shown in this clinical trial that ex vivo whole liver perfusion was capable of carrying out all essential functions of the liver, including excretory (bile and bilirubin excretion), detoxifying (ammonia removal), and synthetic (amino acid and coagulation factor synthesis), for periods of 5 to 12 hours for pig and calf livers, 13 to 24 hours for baboon livers, and 51 hours for ABO-incompatible human livers. Complete recovery of consciousness was achieved in 13 episodes of coma (62%), with significant improvement in consciousness in another 4 (19%). However, with porcine liver perfusion, anti-porcine xenoantibody increased in titer after 1 to 2 weeks, rendering it impossible to use pig livers thereafter. Several other investigators who followed our perfusion criteria also reported successful outcome with porcine liver perfusion in the treatment of hepatic coma as a bridge to transplantation.¹⁶⁻¹⁸

Current Status of Xenogeneic Liver Perfusion

Although the advent of successful liver transplantation and the recent attempts at using bioartificial liver and hepatocyte transplantation have led to reduced interest and experience in extracorporeal liver perfusion therapy, the high incidence of death from liver failure in patients awaiting liver transplantation and the inconsistent success of bioartificial devices have stimulated several centers to revive the technique of ex vivo hepatic perfusion as a bridge to liver transplantation. This has included the use of livers from pigs transgenic for human complement regulatory proteins.¹⁶ Recently, we have modified and redesigned our former ex vivo perfusion system in order to provide a simple and easily assembled

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perfusion circuitry. This includes a specially constructed liver chamber that recreates the normal physiological conditions for the liver, as in vivo, including normal hepatic artery and portal vein inflow pressures, total hepatic blood flow, and liver temperature and oxygen consumption. It consists of a liver chamber with a disposable plastic diaphragm on which the liver rests during perfusion which is made to oscillate by a ventilator at 10 to 15 times per minute in order to prevent hepatic outflow obstruction (Fig. 1 and 2).

Prior to clinical application, a preclinical trial was carried out recently using ex vivo xenogeneic livers from calves to treat dogs with surgically-induced hepatic failure by first performing an end-to-side portacaval shunt under general anesthesia, followed 24 hours later by occlusion of the hepatic artery for 2 hours. We also introduced a very simple and effective method for prior removal of the preformed xenoantibody from the dog by carrying out a temporary kidney transplant from the donor calf before liver perfusion.¹⁹ All animals went into hepatic failure some 8 to 10 hours after occlusion of the hepatic artery. The dogs were divided into two groups. In the first group, control animals (n=6) received only medical treatment. The second group (n=8) was treated with extracorporeal bovine liver perfusion preceded by temporary kidney transplantation from the calf donor to the dog until hyperacute rejection developed after 1-2 hours.

All control animals died in hepatic failure at 14-19 hours following hepatic artery occlusion, while all the animals treated with liver support recovered consciousness, showed marked improvement in their clinical condition and also in all the biochemical liver function parameters. The ex vivo liver continued to function throughout the period of observation (about 8 hours), producing bile with increasing concentration of bilirubin and causing rapid fall in the recipient blood bilirubin from 24 ± 6 to $12 \pm 4 \mu\text{m/L}$. This was accompanied by a significant fall in hepatic enzymes, including alanine aminotransferase (from $4,500 \pm 300$ to $2,400 \pm 200 \mu\text{m/L}$), blood ammonia (from 130 ± 10 to $60 \pm 3 \mu\text{m/L}$), and prothrombin time (from 22 ± 2 to 7 ± 1 seconds). Five of the animals treated recovered completely and became long-term survivors, and the three others survived for 48 to 63 hours. Liver biopsy from the surviving animals showed active liver regeneration. Biopsy of the xenogeneic calf liver taken after perfusion showed only early signs of xenograft

rejection, as manifest by vascular endothelial changes in the portal tract. The relative lack of rejection was due to the removal of the xenoantibody by prior kidney transplantation from calf to dog. Indeed, the titer of lymphocytotoxic antibody level against calf lymphocytes dropped from a mean of 1:512 to 1:4, and the titer of anti-calf thromboagglutinins dropped from 1:128 to 1:16.

Prerequisites for a Successful Liver Perfusion System

The observations and results obtained in this preclinical trial, as well as our previous clinical experience, strongly confirm that extracorporeal perfusion through a xenogeneic liver using the system described would be effective for the support of patients with FHF pending recovery of their own liver or for the long-term support of those who develop acute hepatic decompensation while on the liver transplantation waiting list. However, for the successful use of this ex vivo liver support system, several technical and hemodynamic criteria and prerequisites need to be emphasized.

These include:

1. perfusion of the liver with oxygenated blood through both the hepatic artery and portal vein;
2. maintenance of physiologic pressures in all inflow and outflow hepatic vessels;
3. maintenance of a blood flow rate of about 0.6-0.8 ml/min/gm of liver;
4. maintenance of liver temperature at 37°C ;
5. use of regional heparinization to prevent bleeding problems in the recipient;
6. use of intermittent oscillation of the liver diaphragm to prevent hepatic outflow block;
7. removal of preformed xenoantibodies before perfusion by temporary kidney transplantation from the liver donor or other technique, e.g., plasmapheresis or extracorporeal immunoadsorption through an immunoaffinity column containing synthetic sugars that adsorb the anti-Gal 1-3 Gal antibodies,²⁰ or by using livers from pigs transgenic for a human complement regulatory protein.²¹

From the results of our preclinical trial, we believe that the simplest and most cost-effective method of removing xenoantibody is temporary kidney

... we believe that the simplest and most cost-effective method of removing xeno-antibody is temporary kidney transplantation from the liver donor animal until hyperacute rejection occurs.

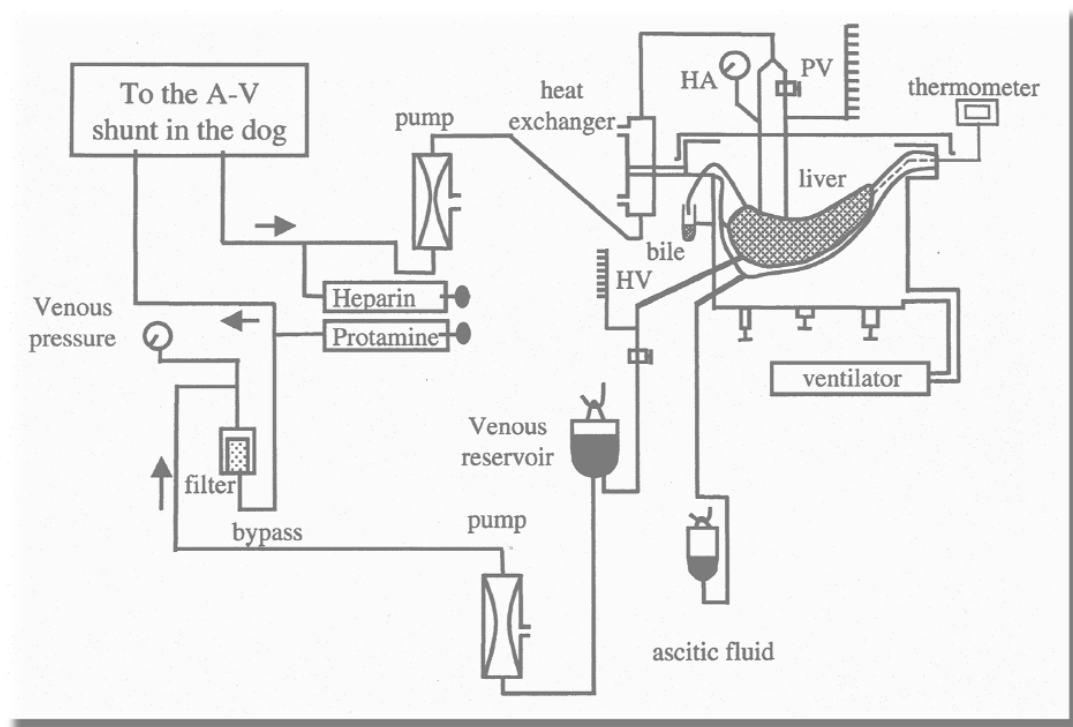


Figure 1. The circuitry of the extracorporeal liver support system.

transplantation from the liver donor animal until hyperacute rejection occurs. Transgenic animals are costly and unnecessary for temporary hepatic support.

Another area where ex vivo liver perfusion has proved useful is in the study of the problems inherent in xenotransplantation. Several centers have used ex vivo liver perfusion to determine the effect of immune manipulation on the function and rate of rejection of the ex vivo liver when perfused with human blood.²²

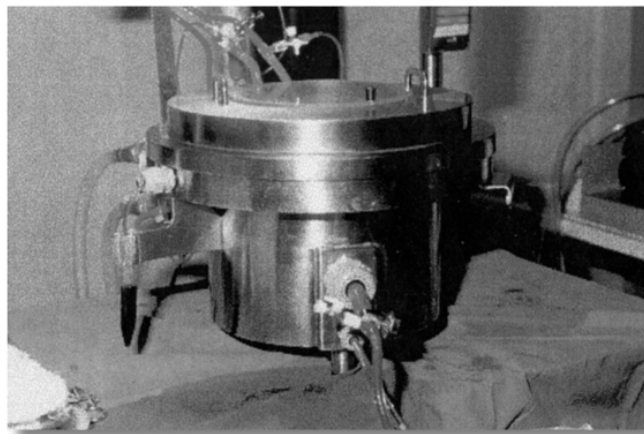
Comment

The ex vivo liver perfusion system as described and performed in the previous clinical and preclinical trials would provide a successful and cost-effective therapy for the treatment of patients with acute, but reversible, hepatic failure as well as for patients awaiting liver transplantation. We would advocate that it should be more widely used, especially when methods of preformed antibody depletion are employed. The need for revitalizing this technique is particularly important at the present time, not only

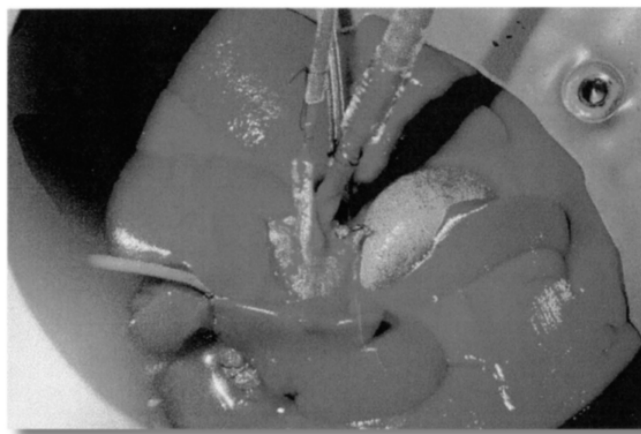
because of the large number of patients who die every year from potentially reversible acute viral or toxic hepatitis, but also because of the increasing number of potential liver transplantation recipients who die while on the waiting list due to the ever-widening gap between organ supply and demand. For these reasons, we strongly believe that the time has come for this form of liver support technology to be re-introduced and used widely in institutions with a major interest in the treatment of patients with hepatic failure and in liver transplantation. It is clearly important that the animals used for ex vivo liver support should be bred and housed in an environment that will prevent or minimize potential disease transmission, although to date several studies have shown no evidence of retroviral transmission to patients using temporary extracorporeal support with pig organs or following porcine islet transplantation.^{23,24}

The current practice of carrying out emergency liver transplantation, using human livers, for patients with FHF (viral or drug-induced hepatitis) is unjustifiable since such patients can be effectively

The current practice of carrying out emergency liver transplantation, using human livers, for patients with FHF ... is unjustifiable since such patients can be effectively and successfully supported by intermittent ex vivo liver perfusion until their own liver regenerates.



The Abouna Chamber



The Liver in the Abouna Chamber

Figure 2. Photograph of the liver perfusion apparatus during xenogeneic extracorporeal liver perfusion (calf-to-dog) showing the calf liver within the chamber (4 hours after the beginning of perfusion).

and successfully supported by intermittent ex vivo liver perfusion until their own liver regenerates. Many of them will not require the complex, expensive and high-risk procedure of undergoing liver transplantation with its associated long-term immunosuppression. Furthermore, emergency liver transplantation is increasingly being performed by the transplantation of part of the liver from a living donor, which involves significant risk to a healthy individual.^{25,26}

It is unlikely that the currently available bioartificial liver devices, containing isolated xenogeneic hepatocytes, will be able to provide the necessary complete hepatic support that can be obtained by xenogeneic whole liver perfusion. Many investigators believe that the bioartificial liver devices, containing 100 to 150 grams of semi-viable encapsulated porcine hepatocytes (instead of the 1500 grams of a normal adult liver), cannot substitute for the myriad of complicated and essential functions of a whole liver. These include the removal of pro-inflammatory

cytokines and the provision of the essential growth factors that aid hepatic regeneration. Clearly, a final and definitive conclusion to this controversy will be obtained only through a well-designed and controlled trial comparing the bioartificial liver cartridge with whole liver perfusion, either in an experimental animal model of acute, but potentially reversible, hepatic failure (as we have described) or in actual patients with FHF in a multi-center project.

Finally, we recall the words of the great physiologist, Claude Bernard (1813-1878), who rightly said: "When the human liver fails, only another normal liver can take its place." We must also remember that "... while a bridge to liver transplantation is good, a bridge to liver regeneration is far better."¹⁸

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