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Hepatocyte transplantation for liver-based metabolic disorders

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Summary Hepatocyte transplantation is being investigated as an alternative to orthotopic liver transplantation in patients with liver-based metabolic disorders. The progress made in this field to date is reviewed. Protocols have been developed using collagenase perfusion to isolate human hepatocytes from unused donor liver tissue. Hepatocytes with a high viability can often be obtained and can be cryopreserved for later use, though with loss of function on thawing. For clinical use, hepatocytes must be prepared in clean GMP conditions with cells meeting criteria of function and lack of microbial contamination before patient use. Hepatocytes are infused intraportally into the patient's liver, where a proportion of cells will engraft and replace the deficient metabolic function without the need for major surgery. Twenty patients have now received hepatocyte transplantation, including eight children at King's College Hospital. There was a range of aetiologies of liver disease: familial hypercholesterolaemia, Crigler–Najjar syndrome type 1, urea cycle defects, infantile Refsum disease, glycogen storage disease type Ia, inherited factor VII deficiency and progressive familial intrahepatic cholestasis type 2. Clinical improvement and partial correction of the metabolic abnormality was observed in most cases. Considerable progress has been made in developing the technique, but hepatocyte transplantation is limited by the available supply of liver tissue. Hepatocytes derived from stem cells could provide alternative sources of cells in the future.

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Introduction

Liver transplantation is the accepted method of treatment for liver-based metabolic disorders. Advances in surgical techniques now allow the use of auxiliary liver transplantation in the management of patients with liver metabolic defects such as Crigler–Najjar syndrome type I, urea cycle defects, and familial hypercholesterolaemia. The success of auxiliary liver transplantation in humans (Pereira et al 1997) has supported the observation in animal experiments that relatively small amounts of liver tissue can provide sufficient function to correct the underlying metabolic defects. This has further increased the interest in using human hepatocytes for cell transplantation in the management of liver-based metabolic conditions.

There are a number of potential advantages of hepatocyte transplantation if the technique can be proved successful. It avoids the risks and undertaking of major surgery and, as the native liver is still in place, it can help bridge a patient to whole-organ transplantation or leave the option of gene therapy, if and when it becomes available in future. Hepatocyte transplantation has been used as a treatment for liver-based metabolic diseases such as Crigler–Najjar syndrome type I (Fox et al 1998), glycogen storage disease type 1a (Muraca et al 2002), urea cycle defects (Horslen et al 2003) and congenital deficiency of coagulation factor VII (Dhawan et al 2004).

Isolation of hepatocytes

There are well-established protocols for isolation of human hepatocytes from unused segments of donor livers (Mitry et al 2002; Strom et al 1998) based on collagenase digestion of cannulated liver tissue at 37°C. After isolation, the hepatocytes are purified by centrifugation and assessed for cell viability and yield. Hepatocytes are better used fresh for cell transplantation as function deteriorates even when they are kept at 4°C for more than 24 h. For longer-term storage, hepatocytes are cryopreserved, but often insufficient viable hepatocytes are recovered on thawing. The best results are currently obtained by cryopreservation in a mixture of the organ preservation medium University of Wisconsin Solution and 10% dimethyl sulphoxide using a controlled-rate cell freezer (Diener et al 1993). The frozen hepatocytes can then be stored at −140°C until required for clinical use.

Sources of liver tissue

The main source of human hepatocytes for transplantation is from unused donor livers and liver segments from which hepatocytes of high viability can be isolated. The quality of cells obtained from livers rejected for clinical transplantation is often not adequate for clinical hepatocyte transplantation (Mitry et al 2003). Over the 5-year period since the start of the project at King's, a total of 120 livers or liver segments have been processed for preparation of hepatocytes. Overall, the mean viability of the hepatocytes obtained was 65%. Newer sources are livers obtained from non-heart-beating donors, marginal grafts (steatotic, liver trauma) and segment IV with or without caudate lobe from split-liver techniques, where one liver is used for two recipients (Mitry et al 2004).

GMP laboratory

An aseptic environment is required for preparation of cells on a large scale in conditions of Good Manufacturing Practice (GMP), so that the isolated cells are safe to be administered to humans. The Cell Isolation Unit is a purpose-built facility consisting of four interconnected rooms. Air entering the laboratory passes through HEPA filters to remove any particles and an air-handling unit maintains a temperature-controlled environment inside the Unit. There is a gradient of air pressures between the rooms, which maintains a positive air pressure differential, with the highest pressure in the Aseptic Room, where the tissue processing is performed. Operators have to wear sterile clean-room suits. Standard operating procedures (SOP) are followed for all aspects of work in the Cell Isolation Unit. A comprehensive quality control system that monitors all aspects of laboratory performance is in operation.

The Cell Isolation Unit at King's is accredited by the Medicines and Healthcare products Regulatory Agency (MHRA) as a tissue bank, being the only one in the UK for isolation of human hepatocytes. Cryopreserved hepatocytes for clinical use are stored in cell freezer bags in the vapour phase of liquid nitrogen inside an automated storage container. The existence of this hepatocyte bank means that hepatocytes are available for immediate use in urgent cases of paediatric liver disease.

All donated organs/tissues are screened for viral infection, including hepatitis and HIV according to the UK Transplant criteria for whole-organ transplantation. The final cell products are screened for microbiological contamination. The hepatocytes can only be released for cell transplantation if the quality is suitable for hepatocyte transplantation (viability $> 60\%$) and no microorganisms are present. To date, 74 isolations have been performed under sterile conditions in the Unit.

Earlier pre-clinical and clinical studies

The reader is directed to excellent recent reviews that fully describe the background to the field of hepatocyte transplantation (Horslen and Fox 2004; Fox and Roy-Chowdhury 2004a,b). A large number of studies carried out in animal models of human liver disease established the feasibility and efficacy of hepatocyte transplantation into various sites such as liver, spleen, pancreas, peritoneal cavity and subrenal capsule. The majority of these studies showed improvement of the biochemical abnormalities, though there were concerns whether sufficient numbers of hepatocytes could be transplanted to normalize function.

The animal studies encouraged human clinical studies of hepatocyte transplantation initially in treatment of patients with acute liver failure. Thirty patients from six centres were reviewed by Strom and colleagues (1999). Hepatocytes, either fresh or after cryopreservation, were infused into the splenic artery or portal vein of patients with liver failure. The number of hepatocytes administered was in the range of 10^7–10^{10} cells. Up to a maximum of 5% of normal liver mass was infused and it is questionable whether this is a sufficient quantity to replace the massive lost function in acute liver failure. In these studies a reduction in ammonia and bilirubin levels and improvements in hepatic encephalopathy were reported.

The cell requirement for transplantation may be lower in inherited metabolic liver diseases, where the aim is to replace a single deficient enzyme. One of the key early reports was from Fox and colleagues in 1998 (Fox et al 1998), who reported treatment of a 10-year-old girl with Crigler–Najjar

Table 1 Hepatocyte transplantation—clinical studies in liver-based metabolic diseases

Liver disease	No. of patients	Effect/outcome	Reference
Familial hypercholesterolaemia	5[a]	Some reduction in LDL in 3 patients	Grossman et al (1995)
Crigler–Najjar syndrome type I	1	50% reduction in serum bilirubin	Fox et al (1998)
	1	Transient reduction in serum bilirubin	Ambrosino et al (2005)
	2	40% reduction in serum bilirubin	Dhawan et al (unpublished)
Urea cycle defect	1	Some clinical improvement, but died after 42 days	Strom et al (1997)
	1	Lowered blood ammonia and increased protein tolerance	Horslen et al (2003)
	1	No hyperammonaemia plus urea synthesis	Mitry et al (2004)
	1	No hyperammonaemia plus urea synthesis	Stephenne et al (2005)
Infantile Refsum disease	1	Partial correction of metabolic abnormality	Sokal et al (2003)
Glycogen storage disease type Ia	1	No hypoglycaemia on normal diet	Muraca et al (2002)
Inherited coagulation factor VII deficiency	2	80% reduction in recombinant factor VII requirement	Dhawan et al (2004)
	1		Dhawan et al (unpublished)
PFIC 2	2	No clear benefit—fibrosis already present	Dhawan et al (unpublished)

[a] *Ex vivo* gene therapy using autologous hepatocytes.

syndrome type I, and showed that there was sustained stable expression of bilirubin-UDP-glucuronosyltransferase activity in the liver up to 9 months following hepatocyte transplantation. The overall experience of hepatocyte transplantation in liver-based genetic liver disease, mainly in children, including that at King's College Hospital is shown in Table 1.

Clinical hepatocyte transplantation at King's College Hospital

The focus has been on children with liver-based metabolic disorders. Firstly, ABO blood group-compatible hepatocytes that meet our criteria for clinical use are needed. It is best to use fresh cells, but these can be supplemented with thawed cryopreserved cells. For transplantation into the liver, up to 10^9 cells per treatment are infused via the portal vein either through an indwelling catheter into a branch of the inferior mesenteric vein or through one placed transhepatically under radiographic screening. It is essential to monitor effects on the hepatic circulation by measuring the hepatic portal venous pressure to avoid the risk of portal hypertension. The aim is to perform repeat cell infusions to provide approximately 10% of total liver mass. It is currently not possible to determine accurately the proportion of administered cells that survive and engraft into the liver and this is an important area for research. The immunosuppression regimen is similar to that given to whole-organ transplantation recipients, currently based on tacrolimus and prednisolone. It is likely that this needs to be further optimized for cell transplantation and methods to determine cell rejection need to be developed.

Once the cell preparation methods in the Cell Isolation Unit were established and validated, the first patient was treated in late 2002. A child with an antenatal diagnosis of ornithine transcarbamylase deficiency received infusion of hepatocytes through an umbilical vein catheter. The clinical course after transplantation of a total of 1.9×10^9 hepatocytes showed a significant improvement in terms of maintenance of blood ammonia levels and an increase in serum urea while the patient was on normal protein diet. Long-term uncertainties about the efficacy of hepatocyte transplantation led to successful auxiliary left lobe orthotopic liver transplantation at 7 months of age. The patient remains well with normal growth and development at 2.5 years of age at the last follow-up.

The next patients were two brothers with severe inherited coagulation factor VII (FVII) deficiency who received

the first use of hepatocyte transplantation in such a condition. Both children received hepatocytes (totals of 1.1 and 2.2×10^9) through a Hickman line inserted in the inferior mesenteric vein. Infusion of isolated human hepatocytes improved the coagulation defect and markedly decreased the requirement for exogenous factor VII (rFVIIa) to ∼20% of that before cell transplantation (Dhawan et al 2004). Importantly, one patient received exclusively cryopreserved hepatocytes, indicating that function, at least related to clotting factors, is maintained after cryopreservation. In both cases, six months after hepatocyte transplantation higher rFVIIa doses were required, suggesting loss of transplanted hepatocyte function, possibly associated with sepsis. With hepatocyte replacement in FVII deficiency there is no selective advantage for the transplanted hepatocytes to repopulate the otherwise healthy host liver. Owing to increasing problems with venous access and long-term uncertainty about the efficacy of hepatocyte transplantation, orthotopic liver transplantation was performed successfully in both cases. Another family member with the same condition has since received hepatocyte transplantation and has shown similar improvement in coagulation parameters with a reduction in factor VII requirement at follow up 3 months after the procedure.

Two other children treated in 2003 were suffering from progressive familial intrahepatic cholestasis (PFIC2), a genetic disease (Thompson and Strautnieks 2001) in which the liver lacks the bile salt export pump (BSEP). As a result of this defect, bile flow is severely impaired and patients rapidly develop liver cirrhosis and need liver transplantation. Both children with PFIC2 received infusion of fresh hepatocytes through a portal vein catheter. Each child received a single infusion of 0.3×10^9 fresh hepatocytes. The rationale was that the injected hepatocytes would have a selective advantage over the patients' own defective hepatocytes to repopulate the native liver, as had been shown in a mouse model of progressive familial intrahepatic cholestasis (De Vree et al 2000), where up to 70% of host hepatocytes were replaced with donor cells. However, both patients had whole-liver transplantation, 5 and 14 months later respectively, as their livers had continued to deteriorate. Existing fibrosis in the hepatic sinusoids is likely to have impaired engraftment of transplanted hepatocytes into the liver structure. Earlier treatment, if feasible, may be the best approach in this situation.

Two children age 18 months and 3 years received hepatocyte transplantation for Crigler–Najjar syndrome. The first patient showed reduction of bilirubin by more than 50% with less phototherapy, but a decrease in function of transplanted hepatocytes was observed after 7 months. His immunosuppression was stopped and he underwent liver transplantation 1 month later. Interestingly, the explant liver showed evidence of engraftment on short tandem repeat analysis for donor DNA and a bile specimen obtained at the operation contained bile conjugates, indicating presence and function of transplanted cells up to 8 months. The 3-year-old girl who underwent hepatocyte transplantation for Crigler–Najjar syndrome in July 2005 has also shown a decrease in bilirubin by 30% and is currently under follow-up on immunosuppression.

The future

Considerable progress has been made in bringing hepatocyte transplantation to the bedside. However, the promise of hepatocyte transplantation from animal experiments performed in ideal experimental conditions usually without the need for immunosuppression has not yet been fully borne out. There are a number of areas for improvement and development.

In terms of the limited supply of livers currently available to isolate hepatocytes, fatty livers are those most commonly rejected for clinical transplantation and represent an important potential source of hepatocytes. Thus improvement of the outcome of isolation and purification of hepatocytes from fatty livers is an important goal, so that these cells could be used for transplantation. There is a need both to improve the storage of hepatocytes for longer periods in the cold so they can be used fresh after a number of days and also for better cryopreservation protocols for longer-term storage. Some progress has been made in our laboratories in understanding the mechanisms of hepatocyte freezing damage and preventing the loss of hepatocyte function after cryopreservation by development of protocols incorporating cryo/cytoprotectant agents (Terry et al 2006). It is clear that many injected cells do not engraft into the recipient liver and are either cleared by the reticuloendothelial system or lose viability during this early phase. The outcome of hepatocyte transplantation would benefit from methods to enhance engraftment and subsequent repopulation of the liver, although the options for this in humans will be limited.

There is no doubt that stem cells/stem cell-derived hepatocytes should offer the potential to overcome the current limitations of both supply of hepatocytes and the extent of repopulation of the liver after transplantation (Fausto 2004). Sources of stem cells for therapy could be fetal livers, cord blood, embryos, and possibly bone marrow. There is a focus of research worldwide on liver stem cell biology and there is no doubt that there are many hurdles to cross before clinical application will be possible. If these are overcome, then stem cells could be differentiated into all types of liver cells, be easier to cryopreserve and thaw with good function, have proliferative capacity *in vitro* and *in vivo*, and may be less immunogenic. As another approach, hepatocytes could be genetically manipulated *in vitro* to upregulate gene expression to enhance enzymatic activity and function (e.g. of ornithine transcarbamylase, bilirubin glucuronyltransferase) or

possibly to render them immune-tolerant. Methods to transfect hepatocytes are available and those using nonviral vectors are of particular interest as they avoid risks due to the use of viral vectors.

In summary, considerable experience has been gained so far in the handling of hepatocytes and techniques for hepatocyte transplantation, allowing clinical hepatocyte transplantation. This will give a good basis for the future application of new technologies, particularly those based on stem cells, which it is hoped will increase the utilization of cell transplantation.

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