Hepatocyte transplantation for metabolic disorders, experience at King's College hospital and review of literature

A. Dhawan, R. R. Mitry, R. D. Hughes

Institute of Liver Studies, King's College London School of Medicine and King's College Hospital, London

Abstract

Hepatocyte transplantation is emerging as a potential treatment for liver based metabolic disorders and acute liver failure. To date, clinical studies have shown that hepatocyte transplantation could be used as a "bridge" to liver transplantation in a few patients with acute liver failure and has changed the phenotype of metabolic patients in terms of reducing the severity of illness. For many years, research studies have been carried out to optimise conditions for a) hepatocyte isolation in order to obtain the highest possible number of viable hepatocytes isolated from various sources of unused donor liver tissues, and b) cryopreservation and storage of hepatocytes for immediate cell availability in emergency cases. In this review, we summarise the worldwide clinical experience in hepatocyte transplantation for liver based metabolic disorders including the experience of our centre at King's College Hospital, London (United Kingdom). We briefly comment on the possible future developments and improvements needed in this field (Acta gastroenterol. belg., **2005**, 68, **457-460**).

Introduction

Advances in medicine have significantly changed the outcome of liver-based metabolic disorders. Development of new drugs like NTBC for tyrosinemia and enzyme replacement therapies for lysosomal disorders are now being increasingly used. For several disorders liver transplantation is now the accepted method of treatment . Advances in surgical techniques, now allow the use of auxiliary liver transplantation in the management of patients with liver-based metabolic defects such as Crigler-Najjar syndrome type I, urea cycle defects, and familial hypercholesterolaemia. The success of auxiliary liver transplantation in humans (1) 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 can help bridge a patient to whole organ transplant or leave the option of gene therapy if and when it becomes available in the future. Hepatocyte transplantation has been used as a treatment for liver based metabolic diseases such as Crigler-Najjar syndrome type I (2), glycogen storage disease type 1a (3), and urea cycle defects (4,5) and congenital deficiency of coagulation factor VII (6).

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 sub-renal capsule. The majority of these studies showed improvement of the biochemical abnormalities, but there were concerns whether sufficient number of hepatocytes could be transplanted to normalise function.

The animal studies encouraged human clinical studies of hepatocyte transplantation initially in treatment of patients with acute liver failure. Thirty patients from 6 centres were reviewed by Strom et al (7). Hepatocytes were infused either fresh or after cryopreservation into the splenic artery or portal vein of patients with liver failure. The number of hepatocytes administered was in the range of 10^7 to 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 (2), who reported treatment of a 10 year old girl with Crigler-Najjar syndrome type I, which showed there was sustained stable expression of bilirubin-UDP-glucuronosyltransferase activity in the liver up to 9 months post-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 the table.

Clinical Hepatocyte Transplantation at KCH

The focus has been on children with liver-based metabolic disorders. Firstly ABO blood group compatible hepatocytes which meet our criteria for clinical use

Correspondence : Prof. Anil Dhawan, MD FRCPCH, King's College London School of Medicine, Paediatric Liver Center, King's College Hospital, Denmark Hill, London SE5 9RS.
E-mail : anil.dhawan@kcl.ac.uk

Table. — **Hepatocyte transplantation for liver based metabolic defects- clinical studies**

Liver disease	No. of patients	Effect/Outcome	Reference
Familial hypercholestrolaemia	5*	Some reduction in LDL in 3 patients	Grossman *et al.* (8)
Crigler-Najjar syndrome type I	1 1 2	50% reduction in serum bilirubin Transient reduction in bilirubin 40% reduction in serum bilirubin	Fox *et al.* (2) Ambrosino *et al.* (9) Dhawan *et al.* (unpubl)
Urea cycle defect	1 1 1 1	Some clinical improvement. Died after 42 days Lowered blood ammonia and increased protein tolerance No hyperammonaemia plus urea synthesis	Strom *et al.* (7) Horslen *et al.* (4) Mitry *et al.* (5) Stephenne *et al.* (10)
Infantile Refsum's disease	1	Partial correction of metabolic abnormality	Sokal *et al.* (11)
Glycogen storage disease type Ia	1	No hypoglycaemia on normal diet	Muraca *et al.* (3)
Inherited coagulation Factor VII deficiency	2 +1	80% reduction in recombinant factor VII requirement	Dhawan *et al.* (6)
PFIC 2	2	No clear benefit – fibrosis already present	Dhawan *et al.* (unpubl)

**ex-vivo* gene therapy of autologous hepatocytes.

are needed. It is best to use fresh cells, but these can be supplemented with thawed cryopreserved cells. For transplantation into the liver up to10^9 cells per treatment are infused via the portal vein either through an in dwelling catheter into a branch of the inferior mesenteric vein or one placed transhepatically under X-ray 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 the proportion of administered cells with accuracy, which 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 optimised for cell transplantation and methods to determine cell rejection developed.

Once the cell isolation 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 billion hepatocytes showed a significant improvement in terms of maintenance of blood ammonia levels, and an increase in serum urea, while 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 (5).

The next patients were two brothers with severe inherited coagulation factor VII deficiency who received the first use of hepatocyte transplantation in such a condition. Both children received hepatocytes (a total of 1.1 and 2.2 billion) 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 (6). Importantly, one patient received exclusively cryopreserved hepatocytes, indicating that function, at least related to clotting factors, is maintained after cryopreservation. Six months post-hepatocyte transplantation in both cases 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. Due to increasing problems with venous access and long-term uncertainty of the efficacy of hepatocyte transplantation, orthotopic liver transplantation was performed successfully in both cases. Another family member has since received hepatocyte transplantation and has shown similar improvement in coagulation parameters with reduction in factor VII requirement at follow up of 3 months after the procedure.

Two other children treated in 2003 were suffering from progressive familial intrahepatic cholestasis (PFIC2) a genetic disease (12) where the liver is lacking 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 one third of a billion fresh hepatocytes. The rationale was that the injected hepatocytes would have a selective advantage over their own defective hepatocytes to repopulate the native liver, as had been shown in a mouse model of progressive familial intrahepatic cholestasis (13), where up to 70% of host

hepatocytes were replaced with donor cells. However, both patients had a whole liver transplant 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 have received hepatocyte transplantation for Crigler-Najjar syndrome. The first patient showed reduction of bilirubin by more than 50% with reduction in phototherapy requirement but decrease in function of transplanted hepatocytes was observed after 7 months. His immunosuppression was stopped and he received liver transplant 4 months later. Interestingly the explant liver showed evidence of engraftment on short tandem repeat assay for donor DNA and a bile specimen obtained at the operation showed bile conjugates indicating the presence and function of transplanted cells up to 8 months. A three year old girl who underwent hepatocyte transplantation for Crigler-Najjar syndrome 2 months back has also shown 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 (14) from fatty livers is an important goal, so that these cells could be used for transplantation. There is a need to improve the storage of hepatocytes, both for longer periods in the cold so they can be used fresh after a number of days and also better cryopreservation protocols for longer term storage (15). 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/cytoprotective agents (16). 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 man 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 (17,18). Sources of stem cells for therapy could be foetal 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 eg. ornithine transcarbamylase, bilirubin glucuronyltransferase or possibly render them immune-tolerant. Methods to transfect hepatocytes are available and those using non-viral vectors are of particular interest as they avoid risks due to 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 utilisation of cell transplantation.

Acknowledgements

We would like to thank the Children's Liver Disease Foundation and King's College Hospital Charitable Trust for financial support. This work would not have been possible without the contributions of Professor Nigel Heaton and Mr Mohamad Rela (transplant surgeons), Dr John Korani (consultant radiologist), together with the other members of the liver transplant surgical team and transplant coordinators at KCH.

References

1. PEREIRA S. P., MCCARTHY M., ELLIS A. J., WENDON J., PORTMANN B., RELA M., HEATON N., WILLIAMS R. Auxiliary partial orthotopic liver transplantation for acute liver failure. *J Hepatol*, 1997, **26** : 1010-7.
2. FOX I. J., CHOWDHURY J. R., KAUFMAN S. S., GOERTZEN T. C., CHOWDHURY N. R., WARKENTIN P. I., DORKO K., SAUTER B. V., STROM S. C. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. *N Engl J Med*, 1998, **338** : 1422-6.
3. MURACA M., GERUNDA G., NERI D., VILEI M. T., GRANATO A., FELTRACCO P., MERONI M., GIRON G., BURLINA A.B. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. *Lancet*, 2002, **359** : 317-8.
4. HORSLEN S. P., MCCOWAN T. C., GOERTZEN T. C., WARKENTIN P.I., CAI H. B., STROM S .C., FOX I. J. Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. *Pediatrics*, 2003, **111** : 1262-7.
5. MITRY R. R., DHAWAN A., HUGHES R. D., BANSAL S., LEHEC S., TERRY C., HEATON N. D., KARANI J. B., MIELI-VERGANI G. and RELA M. One liver, three recipients - segment IV from split liver procedures as a source of hepatocytes for cell transplantation. *Transplant*, 2004, **77** : 1614-1616.
6. DHAWAN A., MITRY R. R., HUGHES R. D., LEHEC S., TERRY C., BANSAL S., ARYA R., WADE J. J., VERMA A., HEATON N. D., RELA M. and MIELI-VERGANI G. Hepatocyte transplantation for inherited factor VII deficiency. *Transplant*, 2004, **78** : 1812-14.
7. STROM S. C., FISHER R. A., THOMPSON M. T., SANYAL A. J., COLE P. E., HAM J. M., *et al*. Hepatocyte transplantation as a bridge to

orthotopic liver transplantation in terminal liver failure. *Transplant*, 1997, **63** : 559-69.
8. GROSSMAN M., RADER D. J., MULLER D. W., KOLANSKY D. M., KOZARSKY K., CLARK B.J. *et al*. A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. *Nat Med*, 1995, **1** : 1148-54.
9. AMBROSINO G., VAROTTO S., STROM S.C., GUARISO G., FRANCHIN E., MIOTTO D., CAENAZZO L., BASSO S., Carraro P., VALENTE M. L., D'AMICO D., ZANCAN L., D'ANTIGA L. Isolated hepatocyte transplantation for Crigler-Najjar syndrome type 1. *Cell Transplant*, 2005, **14** : 151-7.
10. STEPHENNE X., NAJIMI M., SMETS F., REDING R., DE VILLE DE GOYET J., SOKAL E. M. Cryopreserved liver cell transplantation controls ornithine transcarbamylase deficient patient while awaiting liver transplantation. *Am J Transplant*, 2005, **5** : 2058-61.
11. SOKAL E. M., SMETS F., BOURGOIS A., VAN MALDERGEM L., BUTS J. P., REDING R., *et al*. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease : technique, safety, and metabolic follow-up. *Transplant*, 2003, **76** : 735-8.
12. THOMPSON R., STRAUTNIEKS S. BSEP : function and role in progressive familial intrahepatic cholestasis. *Semin Liver Di*s, 2001, **21** : 545-50.
13. DE VREE J. M., OTTENHOFF R., BOSMA P. J., SMITH A. J., ATEN J., OUDE ELFERINK R. P. Correction of liver disease by hepatocyte transplantation in a mouse model of progressive familial intrahepatic cholestasis. *Gastroenterol*, 2000, **119** : 1720-30.
14. STROM S. C., DORKO K., THOMPSON M. T., PISAROV L.A., NUSSLER A. K. Large scale isolation and culture of human hepatocytes. In : Franco D, Boudjema K, Varet B, eds. Îlots de Langerhans et hépatocytes. Paris : Les Editions INSERM, 1998 : 195.
15. MITRY R. R., HUGHES R. D., DHAWAN A.. Progress in human hepatocytes : isolation, culture and cryopreservation. *Sem Cell Dev Biol*, 2002, **13** : 463-7.
16. TERRY C., MURPHY P., MITRY R. R., HUGHES R. D., DHAWAN A. The effect of cryopreservation on the expression of adhesion molecules in human hepatocytes. Abstracts of 7th International Congress of the Cell Transplant Society, Boston, USA, 17-20 Nov 2004.
17. FAUSTO N. Liver regeneration and repair : hepatocytes, progenitor cells, and stem cells. *Hepatol*, 2004, **39** : 1477-8.
18. GERLACH J. C. Prospects of the use of hepatic cells for extracorporeal liver support. *Acta Gastroenterol Belg*, 2005, **68** : 358-368.