Hepatocyte transplantation

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ABSTRACT

Over the last decade the interest in hepatocyte transplantation has been growing continuously and this treatment may represent an alternative clinical approach for patients with acute liver failure and liver-based metabolic disorders.

INTRODUCTION

Orthotopic liver transplantation (OLT) is currently the treatment of choice for end stage liver diseases and liver-based metabolic disorders. The replacement of the diseased organ by OLT is curative, but carries surgical risks and life-long immunosuppressive therapy. Moreover the increasing shortage of donor organs for OLT encouraged the research for alternative therapies for liver diseases. The concept from animal experiments that relatively small amounts of liver tissue can provide sufficient function to correct the underlying metabolic defects was confirmed by the success of auxiliary liver transplantation in the management of patients with acute liver failure and certain liver-based metabolic disorders[1]. This has further increased the interest in using human hepatocytes for cell transplantation in the management of liver-based metabolic conditions and acute liver failure.

HEPATOCYTE TRANSPLANTATION

There are several advantages in the concept of hepatocyte transplantation. It is less expensive and less invasive than OLT, as liver cells can be transplanted after radiological or surgical placement of a portal catheter. Unlike whole organs, hepatocytes can be cryopreserved and stored in cell banks, offering the advantage of immediate availability in emergencies. Theoretically, transplanted cells can functionally replace the hepatocytes of the diseased organ and restore its metabolic capacity either for a period of bridging to whole organ transplantation or by engraftment and long term function. Moreover, the native liver is preserved that leaves the possibility of gene therapy for metabolic disorders open as soon as it becomes clinically available, releasing the recipient from life-long immunosuppressive therapy.

METHODS FOR ISOLATION OF HUMAN HEPATOCYTES

Source of liver tissues

The major obstacle of hepatocyte transplantation is the limited supply of donor liver tissue for cell isolation. Normally liver tissues that become available for hepatocyte isolation have been rejected for conventional OLT, and consequently are of marginal quality. As a result, hepatocytes isolated from these livers are of a low quality and viability. It seems unlikely that this situation will change in the near future. It has been difficult to argue that an already limited donor pool should be shared between a still experimental programme and an established whole organ transplantation programme. However, a few alternatives exist. Cell isolation can be performed in remnants of the liver after orthotopic transplantation of reduced or split liver graft (segment IV), with a significant higher cell viability obtained from these tissues when compared to those rejected for OLT[2,3]. Additionally other alternative sources of hepatocytes are being studied, such as immortalized cell lines[4,5], foetal hepatocytes[6], and stem cell derived hepatocytes[7-9].

Hepatocyte isolation

There are well-established protocols for isolation of human hepatocytes based on collagenase digestion of perfused liver tissue at 37 °C[2,10]. Once the liver tissue is digested and cells released, the hepatocytes are separated by low speed centrifugation, and the pellets obtained are washed with ice-cold buffer solution to purify the cells. Cell viability and yield are then determined. Isolated hepatocytes need to be used as soon as possible for cell

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transplantation, preferably within 24 h of isolation, as function deteriorates even when kept at 4 ∞C. For longer-term storage, human hepatocytes are cryopreserved in a mixture of the organ preservation media University of Wisconsin solution and final concentration of 10% dimethyl sulphoxide (DMSO) using a controlled-rate cell freezer[11]. Cryopreserved cells can then be stored at below –140 °C until required for clinical use.

PRE-CLINICAL STUDIES

Extensive studies using experimental 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. Identification of transplanted hepatocytes was documented by a number of different methods. Models have included hepatocyte transplantation into Nagase analbuminaemic rats, Gunn rats and dipeptidyl peptidase IV-deficient rats. Engraftment and function of transplanted hepatocytes was confirmed by liver immunohistochemistry, and serum albumin levels and reduction in serum bilirubin levels, in the case of Nagase analbuminaemic and Gunn rats, respectively[12,13]. Another approach used was the infusion of genetically modified donor cells secreting or expressing unique reporter proteins, including the green fluorescent protein for direct identification of transplanted cells[14,15].

Hepatocyte transplantation has been described to improve the survival of animal models with acute liver failure, induced either chemically[16-18] or surgically[19]. A number of animal models have been developed to study human liver-based metabolic disorders. Complete or partial correction of the metabolic abnormality by means of hepatocyte transplantation has been reported in some animal models, including the Gunn rat (model for CN syndrome type I)[20], the Long Evans cinnamon rat (model of Wilson's disease)[21], the hyperuricemic Dalmatian dog[22], among others. However, long-term function of transplanted hepatocytes with correction of the underlying metabolic defect has not been conclusively demonstrated. Complete correction of the biochemical abnormality required a significant amount of cells of engrafted cells. Repeated hepatocyte transplantation has shown to increase the number of engrafted liver cells[23], although better results have been seen in animal models where donor hepatocytes have a selective advantage over the native hepatocytes to proliferate and repopulate the recipient liver[20,24].

CLINICAL HEPATOCYTE TRANSPLANTATION

Initial human clinical application of hepatocyte transplantation was for the treatment of patients with acute liver failure (ALF)[25]. These initial trials demonstrated the safety of the technique and some improvement in the outcome of patients. Subsequently, other studies of hepatocyte transplantation for ALF using either fresh or cryopreserved cells have been reported in the literature, showing varying degrees of success[26,27]. Bridging patients to whole-organ transplantation or until recovery of the native liver are the goals of hepatocyte transplantation in ALF.

Currently, the most successful outcome has been for patients with liver-based metabolic disorders. The cell requirement for transplantation may be lower in some inherited metabolic liver diseases where the aim is to replace a single deficient enzyme. The earliest report of patients to receive hepatocyte transplantation for treatment of an inherited liver based metabolic disorder was done by Grossman et al. Five children with familial hypercholesterolemia were transplanted with autologous hepatocytes transduced *ex vivo* with a retroviral vector carrying the human LDL receptor gene. There was evidence of engraftment and over 20% reduction in LDL cholesterol in three of the five patients transplanted, but no sustained expression of the transgene[28,29]. Since then, many other patients have been treated with hepatocyte allotransplantation to correct metabolic diseases.

One of the key early reports is from Fox et al in which the case of a 10-year-old girl with CN syndrome type I treated with hepatocyte transplantation was reported. There was a reduction in her bilirubin levels and hours of phototherapy, and an increase in bilirubin UDP-glucuronosyl transferase activity after hepatocyte transplantation. Long-term evidence of hepatocyte engraftment and function was demonstrated by the excretion of bilirubin conjugates in bile for up to 3.5 years[14,30].

To our knowledge, to date 22 patients with liver-based metabolic disorders were treated with hepatocyte allotransplantation worldwide. Either fresh or cryopreserved cells have been used. At least a transient improvement in bilirubin levels was reported in another four patients with CN type I treated with hepatocyte transplantation (Dhawan et al, unpublished; Allen K, Australia, personal communication)[31]. Six of the other patients treated with hepatocyte transplantation had an urea cycle defect (4 with ornithine transcarbamylase deficiency, 1 with argininosuccinate lyase deficiency, and 1 with citrullinemia)[32-36] (Lee KW, South Korea, personal communication), one an infantile Refsum's disease[37], three a glycogen storage disease type Ia[38] (Lee KW, South Korea, and Sokal EM, Belgium, personal communications), three an inherited coagulation FVII deficiency[39], two a progressive familial intrahepatic cholestasis type 2 (PFIC2) (Dhawan et al, unpublished), and two an α1-antitrypsin deficiency[26,32]. The described outcomes have been variable. The majority of patients experienced partial improvement of their metabolic abnormality, at least for a short period of time. Long-term function of transplanted hepatocytes has also been reported[36,38]. No benefit was observed in the two patients with PFIC2 and the two other ones with α1-antitrypsin deficiency. These patients had already established fibrosis and/or cirrhosis what probably impaired engraftment of the transplanted hepatocytes.

CELL ADMINISTRATION AND SAFETY CONCERNS

The liver and the spleen are the most consistent sites for hepatocyte engraftment and function. Intraportal injection

is the preferred delivery method for clinical hepatocyte transplantation. The portal venous system can be accessed using different techniques: percutaneous transhepatic puncture of the portal vein, transjugular approach to the right portal vein, catheterization of the mesenteric vein or umbilical vein catheterization in newborn babies. Hepatic ultrasound and portal venous system Doppler examination is normally performed before the procedure to exclude any malformation or venous thrombosis, and portal venous pressure is monitored throughout the procedure. Portal hypertension and formation of thrombi of hepatocyte after transplantation appears to be minimized by adding heparin to the cell suspension and limiting the number of cells per infusion to 30-100 $\times$ 10^6/kg of body weight at an infusion rate of 5-10 ml/kg/h, and a concentration of 1-10 $\times$ 10^6 hepatocytes/ml[40]. Thus, when large amounts of hepatocytes need to be injected repeated cell infusions are normally required.

The spleen is considered an adequate site for hepatocyte transplantation, particularly in cirrhotic patients. When injected into the splenic bulb, cells translocate to the liver through the splenic vein. Another attractive site for cell transplantation is the peritoneal cavity due to its large capacity and simple access. Experimental transplantation of encapsulated or matrix attached hepatocytes has prolonged cell survival in animal models[41].

IMMUNOSUPRESSION

To date there is no consensus regarding the immunosuppressive treatment, but most centres have used the protocol of liver transplantation. Combination of tacrolimus and steroids with or without sirolimus or mycophenolate mofetil (MMF) has been used. Some centres use monoclonal antibodies like basiliximab or daclizumab. However, the Edmonton protocol for islet cell transplantation appears to be the most promising.

THE FUTURE

Considerable progress in the field has been made allowing clinical hepatocyte transplantation. However, the success of hepatocyte transplantation from animal models experiments could not be fully reproduced in humans. Although results in clinical studies have been encouraging, no complete correction of any metabolic disease in patients by hepatocyte transplantation alone has been reported. There are still a number of areas for improvement and development. The limited supply and quality of livers currently available to isolate hepatocytes is a major problem for hepatocyte transplantation. Techniques to improve the viability and quality of the cells isolated from marginal livers are required. Another limiting factor of the technique is the conservation and storage of isolated cells. Viability and metabolic capacity on thawing of cryopreserved hepatocytes can be improved by the use of protocols incorporating cryo/cytoprotectant agents[42]. However, there is still 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.

It is also 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 repopulation by induction of a selective growth advantage over host hepatocytes, although the options for this in humans would be limited. Rejection of the allogeneic hepatocytes and/or eventual senescence of the cells transplanted are probably contributing factors for the loss of long-term function of these cells in clinical transplants. Experimental transplantation of hepatocytes encapsulated in semipermeable membranes intraperitoneally in animal models has been shown to maintain long-term viability and function of the cells, without immunosuppression[43]. More studies are needed to minimize or overcome the need of immunosuppression in liver cell transplantation. If tolerance could be achieved, hepatocyte transplantation would exhibit an exceptional advantage over OLT.

It is not likely that the supply of hepatocytes will increase, so a wider use of hepatocyte transplantation will not be possible until alternative sources of cells are found. 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. Foetal hepatocytes, liver stem/progenitor cells isolated from adult livers, embryos, umbilical cord blood and bone marrow, and hepatocytes conditionally immortalized by gene transfer are ongoing areas of investigation. As another approach, autologous hepatocytes could be genetically manipulated *in vitro* to express the missing enzyme. Xenotransplants could be a potentially unlimited source of fresh hepatocytes, however there are many concerns regarding rejection and transmission of infectious diseases that need to be overcome.

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.

REFERENCES

1. Pereira SP, McCarthy M, Ellis AJ, Wendon J, Portmann B, Rela M, et al. Auxiliary partial orthotopic liver transplantation for acute liver failure. J Hepatol. 1997;26:1010-7.
2. Mitry RR, Hughes RD, Aw MM, Terry C, Mieli-Vergani G, Girlanda R, et al. Human hepatocyte isolation and relationship of cell viability to early graft function. Cell Transplant. 2003;12:69-74.
3. Mitry RR, Dhawan A, Hughes RD, Bansal S, Lehec S, Terry C, et al. One liver, three recipients: segment IV from split-liver procedures as a source of hepatocytes for cell transplantation. Transplantation. 2004;77:1614-6.
4. Kobayashi N, Fujiwara T, Westerman KA, Inoue Y, Sakaguchi M, Noguchi H, et al. Prevention of acute liver failure in rats with reversibly immortalized human hepatocytes. Science. 2000;287:1258-62.

5. Cai J, Ito M, Nagata H, Westerman KA, Lafleur D, Chowdhury JR, et al. Treatment of liver failure in rats with end-stage cirrhosis by transplantation of immortalized hepatocytes. Hepatology. 2002;36:386-94.
6. Dan YY, Riehle KJ, Lazaro C, Teoh N, Haque J, Campbell JS, et al. Isolation of multipotent progenitor cells from human fetal liver capable of differentiating into liver and mesenchymal lineages. Proc Natl Acad Sci USA. 2006;103:9912-7.
7. Avital I, Feraresso C, Aoki T, Hui T, Rozga J, Demetriou A, et al. Bone marrow-derived liver stem cell and mature hepatocyte engraftment in livers undergoing rejection. Surgery. 2002;132: 384-90.
8. Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. Stem Cells. 2005;23: 1549-59.
9. Ruhnke M, Ungefroren H, Nussler A, Martin F, Brulport M, Schormann W, et al. Differentiation of in vitro-modified human peripheral blood monocytes into hepatocyte-like and pancreatic islet-like cells. Gastroenterology. 2005;128:1774-86.
10. Strom SC, Dorko K, Thompson MT, Pisarov LA, Nussler AK. Large scale isolation and culture of human hepatocytes. In: Franco D, Boudjema K, Varet B, editors. Îlots de Langerhans et hépatocytes: vers une utilisation therapeutique. Paris: Les Editions INSERM; 1998. p. 195-205.
11. Diener B, Utesch D, Beer N, Durk H, Oesch F. A method for the cryopreservation of liver parenchymal cells for studies of xenobiotics. Cryobiology. 1993;30:116-27.
12. Moscioni AD, Roy-Chowdhury J, Barbour R, Brown LL, Roy-Chowdhury N, Competiello LS, et al. Human liver cell transplantation. Prolonged function in athymic-Gunn and athymic-analbuminemic hybrid rats. Gastroenterology. 1989;96: 1546-51.
13. Gupta S, Rajvanshi P, Lee CD. Integration of transplanted hepatocytes into host liver plates demonstrated with dipeptidyl peptidase IV-deficient rats. Proc Natl Acad Sci USA. 1995;92:5860-4.
14. Horslen SP, Fox IJ. Hepatocyte transplantation. Transplantation. 2004;77:1481-6.
15. Fox IJ, Roy-Chowdhury J. Hepatocyte transplantation. J Hepatol. 2004;40:878-86.
16. Krishna Vanaja D, Sivakumar B, Jesudasan RA, Singh L, Janardanasarma MK, Habibullah CM. In vivo identification, survival, and functional efficacy of transplanted hepatocytes in acute liver failure mice model by FISH using Y-chromosome probe. Cell Transplant. 1998;7:267-73.
17. Sutherland DE, Numata M, Matas AJ, Simmons RL, Najarian JS. Hepatocellular transplantation in acute liver failure. Surgery. 1977;82:124-32.
18. Baumgartner D, LaPlante-O'Neill PM, Sutherland DE, Najarian JS. Effects of intrasplenic injection of hepatocytes, hepatocyte fragments and hepatocyte culture supernatants on D-galactosamine-induced liver failure in rats. Eur Surg Res. 1983; 15:129-35.
19. Demetriou AA, Reisner A, Sanchez J, Levenson SM, Moscioni AD, Chowdhury JR. Transplantation of microcarrier-attached hepatocytes into 90% partially hepatectomized rats. Hepatology. 1988;8:1006-9.
20. Guha C, Parashar B, Deb NJ, Garg M, Gorla GR, Singh A, et al. Normal hepatocytes correct serum bilirubin after repopulation of Gunn rat liver subjected to irradiation/partial resection. Hepatology. 2002;36:354-62.
21. Park SM, Vo K, Lallier M, Cloutier AS, Brochu P, Alvarez F, et al. Hepatocyte transplantation in the Long Evans Cinnamon rat model of Wilson's disease. Cell Transplant. 2006;15:13-22.
22. Kocken JM, Borel Rinkes IH, Bijma AM, De Roos WK, Bouwman E, Terpstra OT, et al. Correction of an inborn error of metabolism by intraportal hepatocyte transplantation in a dog model. Transplantation. 1996;62:358-64.
23. Rozga J, Holzman M, Moscioni AD, Fujioka H, Morsiani E, Demetriou AA. Repeated intraportal hepatocyte transplantation in analbuminemic rats. Cell Transplant. 1995;4:237-43.
24. De Vree JM, Ottenhoff R, Bosma PJ, Smith AJ, Aten J, Oude Elferink RP. Correction of liver disease by hepatocyte transplantation in a mouse model of progressive familial intrahepatic cholestasis. Gastroenterology. 2000;119:1720-30.
25. Habibullah CM, Syed IH, Qamar A, Taher-Uz Z. Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. Transplantation. 1994;58:951-2.
26. Strom SC, Chowdhury JR, Fox IJ. Hepatocyte transplantation for the treatment of human disease. Semin Liver Dis. 1999; 19:39-48.
27. Schneider A, Attaran M, Meier PN, Strassburg C, Manns MP, Ott M, et al. Hepatocyte transplantation in an acute liver failure due to mushroom poisoning. Transplantation. 2006;82: 1115-6.
28. Grossman M, Raper SE, Kozarsky K, Stein EA, Engelhardt JF, Muller D, et al. Successful ex vivo gene therapy directed to liver in a patient with familial hypercholesterolaemia. Nat Genet. 1994;6:335-41.
29. Grossman M, Rader DJ, Muller DW, Kolansky DM, Kozarsky K, Clark BJ, 3rd, et al. A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. Nat Med. 1995;1: 1148-54.
30. Fox IJ, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI, et al. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl J Med. 1998;338:1422-6.
31. Ambrosino G, Varotto S, Strom SC, Guariso G, Franchin E, Miotto D, et al. Isolated hepatocyte transplantation for Crigler-Najjar syndrome type 1. Cell Transplant. 2005;14:151-7.
32. Strom SC, Fisher RA, Rubinstein WS, Barranger JA, Towbin RB, Charron M, et al. Transplantation of human hepatocytes. Transplant Proc. 1997;29:2103-6.
33. Horslen SP, McCowan TC, Goertzen TC, Warkentin PI, Cai HB, Strom SC, et al. Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. Pediatrics. 2003;111(6 Pt 1):1262-7.
34. Tan N, Puppi J, Mitry R, Hughes R, Lehec S, Mieli-Vergani G, et al. Hepatocyte transplantation followed by auxiliary liver transplantation – a novel treatment for ornithine transcarbamylase deficiency. Am J Transplant. In press.
35. Stephenne X, Najimi M, Smets F, Reding R, De Ville de Goyet J, Sokal EM. Cryopreserved liver cell transplantation controls ornithine transcarbamylase deficient patient while awaiting liver transplantation. Am J Transplant. 2005;5:2058-61.
36. Stephenne X, Najimi M, Sibille C, Nassogne MC, Smets F, Sokal EM. Sustained engraftment and tissue enzyme activity after liver cell transplantation for argininosuccinate lyase deficiency. Gastroenterology. 2006;130:1317-23.
37. Sokal EM, Smets F, Bourgois A, Van Maldergem L, Buts JP, Reding R, et al. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. Transplantation. 2003;76:735-8.
38. Muraca M, Gerunda G, Neri D, Vilei MT, Granato A, Feltracco P, et al. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. Lancet. 2002;359:317-8.
39. Dhawan A, Mitry RR, Hughes RD, Lehec S, Terry C, Bansal S, et al. Hepatocyte transplantation for inherited factor VII deficiency. Transplantation. 2004;78:1812-4.
40. Fisher RA, Bu D, Thompson M, Wolfe L, Ritter JK. Optimization of conditions for clinical human hepatocyte infusion. Cell Transplant. 2004;13:677-89.
41. Fox IJ, Chowdhury JR. Hepatocyte transplantation. Am J Transplant. 2004;4 Suppl 6:7-13.
42. Terry C, Dhawan A, Mitry RR, Lehec SC, Hughes RD. Preincubation of rat and human hepatocytes with cytoprotectants prior to cryopreservation can improve viability and function upon thawing. Liver Transpl. 2005;11:1533-40.
43. Balladur P, Crema E, Honiger J, Calmus Y, Baudrimont M, Delelo R, et al. Transplantation of allogeneic hepatocytes without immunosuppression: long-term survival. Surgery. 1995;117: 189-94.