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Concise Review

Hepatocyte transplantation: State of the art and strategies for overcoming existing hurdles*

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Abstract

Over three decades of research in experimental animals and several clinical trials have brought us to the threshold of hepatocyte transplantation for the treatment of acute and chronic liver failure, and inherited metabolic disorders. However, more extensive clinical studies and routine clinical application are hampered by the shortage of good quality of donor cells. To overcome these hurdles, current research has focused on the search for alternatives to adult primary hepatocytes, such as liver cell progenitors, fetal hepatoblasts, embryonic, bone marrow or umbilical cord blood stem cells and conditionally immortalized hepatocytes. Cross-species hepatocyte transplantation is also being explored. It is hoped that ongoing research will permit the application of hepatocyte transplantation to the treatment of a wide array of liver diseases.

Key words: Hepatocytes, transplantation, acute liver failure, chronic liver failure, inherited disorders.

Introduction

Despite the spectacular success of whole or partial liver transplantation in the treatment of acute and chronic liver failure, and inherited metabolic diseases, the technique remains complex, expensive and associated with significant morbidity and mortality. Furthermore, the supply of cadaver donor organs has remained constant for a decade, while demand for transplantable livers is increasing progressively, outpacing the availability of donated cadaver organs.[1] Although the use of adult living donors may abate the organ shortage to some extent, this procedure is not without significant risk to the donor and the recipient.[2] In view of this, many investigators have evaluated transplantation of isolated liver cells as a less invasive alternative to whole organ transplantation or as a "bridge" while awaiting the availability of a donor liver.[1] In contrast to intact livers, hepatocytes could be cryopreserved for immediate availability in emergencies.[3] Since the recipient liver remains intact, the metabolic risk of transplant rejection is minimized and the possibility of subsequent orthotopic liver transplantation or liver-directed gene therapy remains open. This minimally invasive procedure requires minimal or no hospitalization, which should lower the cost of the procedure and permit earlier treatment of inherited or acquired liver disorders, thereby reducing complications of the diseases. Studies on laboratory animals over the last three decades and recent clinical trials indicate the usefulness of liver cell transplantation in the treatment of metabolic liver diseases and as a bridge for patients with liver failure awaiting transplantation. Safety and feasibility of this approach have been demonstrated. However, widespread application of liver cell transplantation has been tantalizingly slow, principally because of the shortage of usable primary human hepatocytes, which is, at this time, even more severe than the shortage of transplantable organs. It is anticipated, therefore, that in the coming years, investigators will focus on identifying alternatives to adult primary hepatocytes for transplantation and methods for inducing selective proliferation of the transplanted cells. A brief discussion of the current issues in liver cell transplantation follows.

Sites of liver cell transplantation: The physiological matrix and portal blood supply make liver the optimum site for engraftment and survival of hepatocytes. When the liver architecture is relatively normal, hepatocytes can be seeded readily into the liver by infusion into the portal vein or injection into the splenic pulp, from where the cells migrate to the liver.[4] The cells leave portal vein branches within a few days and integrate into the liver cord, where they cannot by readily distinguished from the host hepatocytes, unless they possess detectable genetic differences[5] or are marked by some other means. In the

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presence of ongoing liver injury, engraftment efficiency and survival of transplanted hepatocytes is variable. Transplantation of hepatocytes into cirrhotic livers causes prolonged and severe portal hypertension and migration of the injected cells to pulmonary capillary bed through collateral circulation.[6] The search for ectopic sites for hepatocyte engraftment has revealed that the pulmonary capillary bed and subcutaneous tissues do not support prolonged hepatocyte survival. Renal subcapsular sites can accommodate a small number of liver cells. The peritoneal cavity can accommodate a large number of hepatocytes, but as hepatocytes are anchor-dependent, they need to be attached to collagen-coated surfaces[5,7] or be encapsulated in alginate beads. Although the spleen is often used as a conduit for seeding hepatocytes into the liver parenchyma, hepatocytes can also engraft and survive in the splenic pulp, which is particularly useful in recipient animals with portacaval shunt or cirrhosis of the liver. The spleen appears to be a better site for hepatocytes to function than are cirrhotic hepatic nodules.

The mechanism of hepatocyte engraftment in the liver has been investigated in detail. Hepatocytes injected into the spleen or infused into the portal vein are translocated to the liver within seconds to minutes, where they become entrapped in the portal veinules and the periportal sinusoidal space. Relatively large size of hepatocytes, as well as interaction of cell surface proteins with their matrix ligands may responsible for hepatocyte entrapment. Transient portal hypertension resulting from blood flow occlusion resolves in 2-3 hours after transplantation. During the ensuing 3-7 days, the entrapped hepatocytes migrate through the vascular endothelium and become integrated into the liver cords. The specific mechanism of this phenomenon is not fully understood, but transient ischemic damage of the endothelial cells and release of various cytokines, may be involved.[8]

Sources of donor cells: Primary hepatocytes engraft readily and begin providing differentiated function immediately upon engraftment. In mice and rats, engrafted hepatocytes exhibit apparently unlimited regenerative capacity, whereby a small number of cells can repopulate the entire liver.[9,10] However, it is questionable whether adult human hepatocytes, which lack telomerase activity, would have similar proliferative capacity. Generally adult hepatocytes for transplantation are obtained by perfusing cadaver livers that had been considered on insufficient quality for organ transplantation. Cells obtained from such livers are often not of the highest quality and are difficult to cryopreserve. Efforts are underway to isolate subpopulations of hepatocytes from these cells that might permit crypreservation and efficient engraftment, thereby expanding the available pool of donor hepatocytes. Engraftment and survival of hepatocytes transplanted at ectopic sites may be augmented by cotransplantation of non-parenchymal liver cells, which provide the attachment matrix and soluble factors required for growth and differentiated function of hepatocytes.[11]

Fetal hepatocytes derived from abortuses could be an alterative source of donor cells. Fetal rat hepatocytes transplanted into adult rat livers regenerating after 66% hepatectomy, form cell clusters consisting of 500-1000 cells in 2 months, whereas similarly transplanted adult hepatocytes undergo only one or two cell divisions.[12] It will be important to determine whether cells derived from fetal livers before 12 weeks of gestational age, when most elective abortions are performed, would express differentiated hepatocellular function after transplantation.

The liver contains a small pool of stem-like cells that expand in response to proliferative stimuli in situations where the regenerative capacity of mature hepatocytes is impaired.[13] Such cells, isolated from the liver or pancreas, can differentiate into hepatocytes and bile duct epithelial cells. However, current methods do not permit expansion of these progenitor cells in culture to numbers that are large enough for clinical application.

Stem or progenitors cells capable of giving rise to hepatocytes have been sought also in non-hepatic tissues. Genetic or enzymatic markers of donor bone marrow cells have been detected in the liver after bone marrow transplantation.[14,15] Initially these results had suggested that bone marrow stem cells trans-differentiate into hepatocytes. Subsequent studies indicated that the findings could have resulted from fusion of donor bone marrow stem cells with recipient hepatocytes.[16] Effort is also underway to generate hepatic progenitor cells from embryonic or umbilical cord blood stem cells.[17,18]

Hepatocytes isolated from a resected liver segment of a mutant subject could be phenotypically corrected by gene transfer, and then transplanted back into the donor. This process, termed ex vivo gene therapy, was used to reduce plasma low-density lipoprotein (LDL) levels in LDL receptor-deficient rabbits.[19] As the donor and the recipient are the same, immune suppression is not needed. However, as discussed later, a clinical trial based on this strategy failed to produce a therapeutically significant clinical response in patients with familial hypercholesterolemia,[20] probably because of low transduction efficiency of the gene transfer vector.

The shortage of high quality primary human hepatocytes has prompted investigators to generate non-tumorigenic immortalized hepatocytes by transferring genes, such as simian virus 40 T antigen (Tag), the active subunit of human telomerase (hTERT), cyclin D1 and a constitutively active hepatocyte growth factor receptor (truncated cMet).[21-23] Although these genes do not transform cells by themselves, a "second hit", such as activation of a proto-oncogene, can lead to malignant transformation. To reduce this risk, hepatocytes have been immortalized conditionally, using genes that express a thermolabile mutant form of Tag, which is degraded at body temperature. In other cases, the Tag gene was flanked by *lox* se-

quences, which permitted its excision by expressing Cre recombinase. Suicide genes, e.g. HSV thymidine kinase have been included in the gene transfer construct in some cases, so that the cells can be killed using the pro-drug ganciclovir, in the eventuality of malignant transformation of the transplanted immortalized cells. Conditionally immortalized hepatocytes have been transplanted to successfully rescue rats that had undergone 90% hepatectomy[23] and to provide ammonia detoxification in portacavally shunted or cirrhotic rats.[24] Conditional immortalization could allow expansion of a small number of autologous cells obtained by liver biopsy. During rapid expansion, the cells could be transduced efficiently with therapeutic genes, facilitating ex vivo gene therapy.

Parallel to the efforts to use animal livers for cross-species transplantation, porcine livers have been explored as sources of donor hepatocytes for cross-species transplantation. Hyperacute rejection, mediated by natural killer cells and complement-based mechanisms remains a major problem in cross-species organ transplantation. Surprisingly, however, porcine hepatocytes, transplanted into cirrhotic rats, were tolerated well with standard immunosuppressive protocols.[25] If these results could be reproduced in primate models, non-human livers could be a potential source of high quality hepatocytes for clinical use.

Hepatocyte transplantation in animal models of liver failure

Since the normal hepatic architecture remains intact in most cases of acute liver failure, hepatocytes may be expected to engraft and provide early metabolic support, providing time for the residual host cells to regenerate. Hepatocyte transplantation has been shown to improve the survival of animals with both chemically and surgically induced acute liver failure.[26] Importantly, in pigs with acute, ischemic liver failure, this procedure prevented the development of intracranial hypertension, which is a major cause of death in acute liver failure.[27] However, in most animal models studied, regeneration of the host liver is not inhibited, which is in contrast to the situation in human liver failure. Thus, the dramatic results obtained in animal studies could have resulted from a very short-term metabolic support provided by hepatocyte transplantation. Not surprisingly, therefore, hepatocyte transplantation in patients with acute liver failure has succeeded unequivocally only when used as a "bridge" to liver transplantation. This notion is supported by studies in a murine model in which a toxin is used to induce both acute liver failure and inhibition of hepatocyte regeneration.[28] In this model, the results suggested that a single infusion of donor hepatocytes is unlikely to improve survival significantly.

To evaluate the efficacy of hepatocyte transplantation in providing metabolic support in chronic liver failure, primary or immortalized hepatocytes were transplanted into the spleen of rats with end-to-side portacaval shunt. In these models, the cells engraft in the spleen, but cannot migrate to the liver. Hepatocyte transplantation significantly improved their neurobehavioral score and amino acid balance,[29] and prevented hepatic coma following administration of ammonium chloride.[30] Similar improvement in metabolic function and survival was observed in rats with decompensated liver cirrhosis induced with carbon tetrachloride and phenobarbital administration.[31]

Hepatocyte transplantation in animal models of inherited metabolic liver diseases

Rats, mice and rabbits with subtle or serious liver-based metabolic abnormalities have been used to evaluate the efficacy of hepatocyte transplantation. Transplantation of hepatocytes, equivalent to 1-5% of the total hepatocyte mass, resulted in partial correction of hyperbilirubinemia in UGT1A1-deficient Gunn rats and increase of serum albumin levels in Nagase analbuminemic rats.[5,7] In these experiments hepatocytes were attached to collagen-coated microcarriers prior to injection into the peritoneal cavity. Hepatocyte transplantation into the liver, via portal vein infusion or intrasplenic injection, partially ameliorated the abnormality of copper metabolism in the Long-Evans Cinnamon (LEC) rat model of Wilson's Disease.[32] hypercholesterolemia in the LDL receptor-deficient Watanabe hyperlipidemic rabbit (a model of familial hypercholesterolemia)[33] and defective biliary phospholipid excretion in the *mdr2*-knockout mouse model of progressive familial intrahepatic cholestasis type 3.[34]

In rare situations, such as fumaryl acetoacetate hydrolase (FAH)-deficient mouse model of familial tyrosinemia[10] and uPA transgenic mice,[9] host hepatocytes have markedly reduced survival, due to the inherited metabolic defect. In these cases transplanted wild-type hepatocytes spontaneously replace the host cells over time, leading to near-complete repopulation of the liver. Partial repopulation by engrafted hepatocytes occurs in LEC rats and mdr2-deficient mice, where the hepatocellular injury is subtle. However, in the most cases of liver-based inherited diseases, the life-span and regenerative capacity of host hepatocytes are normal, and, therefore, transplanted hepatocytes do not spontaneously repopulate the liver. As a result, it has not been possible to fully correct metabolic diseases by single procedure of hepatocyte transplantation. Although the number of engrafted cells hepatocytes can be increased by repeated transplantation,[35] a much more efficient approach would be to induce preferential proliferation of the transplanted cells over host hepatocytes. This has been achieved in experimental animals by providing a strong proliferating stimulus for hepatocytes, while pre-treating the recipients by a protocol that inhibits host hepatocyte proliferation. In initial studies, rats were treated with plant alkaloids, such as retrorsine, to prevent hepatocellular proliferation, and partial hepatec-

tomy was performed to provide a proliferative stimulus to the engrafted cells.[36] As retrorsine is potentially carcinogenic, other investigators have employed preparative irradiation of the liver, in a manner similar to that used for bone marrow transplantation.[37,38] By this approach, it has been possible to replace over 90% of host hepatocytes with donor cells, resulting in complete correction of hyperbilirubinemia in Gunn rats.[37] Studies in progress focus on substituting the partial hepatectomy with non-invasive approaches, such as the administration of thyroid hormone[39] or other hepatocellular mitotic stimuli.

Clinical studies

Acute liver failure: An early clinical study in patients with acute liver failure showed that injection of human fetal liver cells into the peritoneal cavity resulted in a small but statistically significant improvement in overall survival, compared with age-matched controls, particularly in patients with grade 3 hepatic coma.[40] In later studies, hepatocyte transplantation was used primarily to "bridge" patients with acute liver failure awaiting the availability of a donor liver.[41,42] In general, 10^7 to 10^{10} allogeneic hepatocytes from adult cadaver livers were infused into the splenic artery or the portal vein. There are isolated reports of improvement in serum ammonia levels, prothrombin time, level of encephalopathy, cerebral perfusion pressure and cardiovascular stability. Complications included sepsis and hepatocyte embolization into the pulmonary circulation, and transient, reversible hemodynamic instability.[43] The low level of clinical benefit could have been due to the small number of hepatocytes used and the splenic arterial route of injection, which results in poor hepatocyte survival in animal experiments.

Chronic liver failure: In most patients with hepatic cirrhosis, the cirrhotic nodules contain hepatocytes in large enough numbers that could have been expected to support metabolism at a relatively normal level. However, hepatocytes present in cirrhotic nodules are dysfunctional because of abnormalities of the hepatic architecture. Based on this concept, investigators have transplanted hepatocytes recovered from segments of the cirrhotic livers of patients and transplanted them by injection into the splenic pulp, splenic artery, splenic vein or portal vein.[43,44] Although the injections were tolerated well and there was some evidence of improvement in encephalopathy, protein synthesis and renal function, the ultimate clinical outcome was not altered significantly. In retrospect, the results were not surprising because most of these patients had received hepatocyte transplantation through the splenic artery.[45] Animal experiments show that hepatocytes infused into arterial beds do not survive long-term.[6]

Liver-based inherited metabolic diseases: As discussed above, attempts to treat familial hypercholesterolemia (LDL receptor deficiency) by ex vivo gene therapy did not result in therapeutically significant reduction of serum cholesterol levels.[20] However, these studies demonstrated the safety and feasibiliry of hepatocyte transplantation. Subsequently, other investigators have transplanted allogeneic hepatocytes into the liver bed to correct ornithine transcarbamylase (OTC) deficiency, alpha-1-antitrypsin deficiency, glycogen storage disease type Ia, infantile Refsum disease and Crigler-Najjar syndrome type 1.[45-50] Hepatocyte transplantation resulted in transient correction of hepatic OTC deficiency.[47,48] Long-term improvement in glucose metabolism was reported after hepatocyte transplantation in an adult patient with glycogen storage disease type Ia.[49] Direct evidence of survival and function of transplanted human hepatocytes was obtained in a 10 year old patient with Crigler-Najjar syndrome type I (UGT1A1 deficiency), in whom serum bilirubin levels were reduced to 50% of pretransplant levels, and 5% of hepatic UGT1A1 activity was reconstituted following a single session of hepatocyte transplantation. However, the metabolic correction was not sufficient to eliminate the need for phototherapy. Therefore, although the bile contained bilirubin glucuronides two and a half years after hepatocyte transplantation, indicating persistence of the transplanted hepatocytes, the patient ultimately underwent successful auxiliary liver transplantation.[46] Recently, a 4 year old patient with infantile Refsum disease received hepatocyte transplantation, which led to partial clearance of abnormal bile acids; with pipecholic acid being reduced to 60% of pre-transplantation levels. The child was able to stand and walk 6 months after hepatocyte transplantation.[50]

Ongoing research

To retrieve transplantable hepatocytes from cadaver donor livers that are not accepted for organ transplantation, investigators are attempting to isolate viable hepatocytes from total liver cell isolates, based on the concept that smaller hepatocytes may retain their viability longer during prolonged liver ischemia. Since primary hepatocytes from adult human liver cannot be expanded greatly in culture without genetic modification, reseach has focused on the use of fetal hepatoblast/hepatocytes, liver stem/progenitor cells isolated from adult liver, embryonic or umbilical cord blood stem cells and hepatocytes conditionally immortalized by gene transfer. Studies are also underway to explore xenogenic hepatocytes for transplantation. Although concerns about hyperacute xenograft rejection have not been addressed fully, current data indicate that cirrhotic animals may tolerate xenogenic hepatocytes. Providing proliferative advantage to transplanted cells by manipulations of the host liver is an active area of current research. Since long-term immunosuppression is associated with significant risk of toxic injury, genetic manipulation of donor hepatocytes to induce immune ignorance in the host or tolerance to allogeneic or xenogenic hepatocytes is another area of active research.

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