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Barriers to the successful treatment of liver disease by hepatocyte transplantation

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Introduction

The challenge of treating life-threatening liver disease by transplantation of isolated hepatocytes

Management of patients with hepatic failure and liver-based metabolic disorders is complex and expensive. Hepatic failure results in impaired coagulation, altered consciousness and cerebral function, a heightened risk of multiple organ system failure, and sepsis [1]. Such manifold problems are only treatable today and for the foreseeable future by transplantation. In fact, whole or auxiliary partial liver transplantation is often the only available treatment option for severe, even if transient, hepatic failure. Patients with life-threatening liver-based metabolic disorders similarly require organ transplantation even though their metabolic diseases are typically the result of a single enzyme deficiency, and the liver otherwise functions normally. For all of the benefits it may confer, liver transplantation is not an ideal therapy, even for severe hepatic failure. More than 17,000 patients currently await liver transplantation in the United States, a number that seriously underestimates the number of patients that need treatment [2], as it has been estimated that more than a million patients could benefit from transplantation [3]. Unfortunately, use of whole liver transplantation to treat these disorders is limited by a severe shortage of donors and by the risks to the recipient associated with major surgery [4].

Hepatocyte transplantation holds great promise as an alternative to organ transplantation for the treatment of liver diseases, and numerous studies in rodents indicate that transplants consisting of isolated liver cells can correct various metabolic deficiencies of the liver and can reverse hepatic failure [5–17]. The transplant procedure, which involves injection of isolated hepatocytes into the liver or spleen, is far less invasive than transplantation of the whole liver and could be performed on severely ill patients with relatively low risk. In the presence of normal host liver architecture, the transplanted cells integrate into the host liver, providing considerable restorative potential [18]. Because the native liver is not removed, the transplanted hepatocytes need only improve some of the functions of the failing liver and need not replace all hepatic functions.

Although clinical trials of hepatocyte transplantation have demonstrated the long-term safety of the procedure, only partial correction of metabolic disorders has been achieved, and the degree to which donor hepatocytes have restored failing livers has not generally been adequate to circumvent the need for organ replacement [19–27]. While hepatocyte transplantation can be employed safely in humans, its applicability remains limited by a number of issues, some of which include: (1) a critical shortage of donor organs and hepatocytes for transplantation; (2) relatively poor initial and long-term hepatocyte engraftment that limits successful treatment of chronic diseases, such as liver-based metabolic deficiencies; and (3) the lack of a clinically relevant animal model of acute liver failure that could be used to accurately predict the efficacy of new therapies in treating this process.

The challenge of treating acute liver failure

Several thousand cases of acute liver failure occur each year in the United States. Approximately 40% of patients with advanced symptoms survive the acute episode with only medical management. In these cases, regeneration of the native liver makes orthotopic liver

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Abbreviations: ECM, extra-cellular matrix; ES cells, embryonic stem cells; IMRT, intensity-modulated radiation therapy; iPS cells, induced pluripotent stem cells.

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transplantation unnecessary. Unfortunately, there is no effective means to distinguish between patients who will survive without transplantation from those who will not. Support options exist for patients with acute renal or cardiovascular insufficiency, obviating the need for transplantation. Unfortunately no effective support exists for patients with liver failure.

Several factors have hindered the development of innovative therapies for treating patients with fulminant liver failure. Despite the availability of numerous surgical and pharmacologic-based animal models of acute hepatic failure, none recapitulate clinical hepatic failure to the point where the efficacy of cell transplantation or liver assist devices could be predicted in patients [28,29]. The severity of liver dysfunction requires that the transplanted hepatocytes function immediately, but the lack of a clinically relevant disease model means that the number of cells that would need to engraft and function immediately to reverse hepatic failure remains essentially unknown. Since clinical experience with auxiliary orthotopic liver transplant for acute hepatic failure indicates that native liver recovery may take 6 months to a year, if ever [30], liver recovery in any representative animal model needs to take at least a week, if not more. The highly variable natural history and the numerous etiologies of acute liver failure have also made assessment of the success of novel interventions in this patient population difficult. Thus, while extracorporeal liver assist devices and hepatocyte transplantation have been applied to the treatment of acute hepatic failure, neither approach has reliably resulted in reversal of hepatic failure to the point where organ transplantation can be avoided [31].

For hepatocyte transplantation, interpretation of its potential has been further confounded by the wide range in the numbers and types of cells transplanted, the sites where cells have been infused, and spontaneous recovery rates approaching 40% [21,27,32–39]. It is possible that enrollment of patients in a multi-institutional standardized treatment protocol would help delineate the potential that transplantation of hepatocytes might have for the treatment of fulminant liver failure, and would help identify any hurdles to its successful application. Such a standardized protocol could focus on trying to infuse $1–2 \times 10^8$ viable hepatocytes per kilogram through the portal vein for engraftment in the liver. Even this may be difficult to accomplish in a multi-center trial, as the coagulopathy associated with acute liver failure makes access to the portal circulation challenging. Fresh or cryopreserved hepatocytes could be considered for such a trial, but because cryopreserved cells have been shown to engraft less well than fresh hepatocytes (discussed later in this review), an aliquot of each population would need to be characterized in an identical fashion, for comparison, for *in vitro* activity, plating efficiency, and engraftment potential in immune deficient hosts.

The challenge of treating chronic liver disease resulting from cirrhosis

Hepatocyte transplantation for end-stage liver disease is even more problematic. Abnormalities in hepatic architecture contribute to decrease in liver function, and transplantation of hepatocytes into the portal vein of a cirrhotic liver can generate severe portal hypertension. Furthermore, it is not clear that donor hepatocytes can function for any sustained period of time to improve hepatic failure in the abnormal cirrhotic environment [24,40]. Animal studies suggest that transplantation into the spleens of rats with decompensated liver cirrhosis can improve liver function, and prolong survival [41,42]. Unfortunately, transplanted hepatocytes provided only transient function. Hepatocyte transplantation in humans with end-stage cirrhosis has not resulted in even this level of success, but only anecdotal improvement in some parameters of liver function [24,27,40,43,44]. One explanation may be that hepatocytes were infused through the splenic artery in clinical studies, rather than by the direct splenic puncture approach used in the laboratory. The route of hepatocyte delivery into the spleen has been shown to dramatically influence hepatocyte engraftment and function [45]. Treatment of chronic liver failure might benefit in the future from a new technology called organ de-cellularization, where the cells from a donor organ are removed, leaving intact the complex mixture of structural and functional proteins that constitute the ECM. A de-cellularized human or animal liver could serve as a biologic, architecturally normal scaffold for transplanted cells [46,47]. The scaffold, repopulated with donor hepatocytes and non-parenchymal cells, might then be vascularized through the portal circulation as an engineered internal auxiliary liver graft [48]. Since hepatocyte transplantation in chronic liver disease will leave the native cirrhotic liver in place, even if successful at improving liver function, it will still leave unresolved the management of coexisting portal hypertension and the risk of developing hepatocellular carcinoma in the native liver (Box 1).

Keypoints

Acute Fulminant Liver Failure

Effective as a bridge to organ transplantation

Barriers:

- Critical shortage of hepatocytes for transplantation
- Large animal models of acute hepatic failure that mimic human disease in man lacking
- The number of hepatocytes needed to treat fulminant liver failure unknown
- Risk of bleeding is significant with percutaneous access to the portal vein for cell infusion
- Workable extrahepatic site for clinical hepatocyte transplantation not yet identified

Possible areas for progress:

- In the absence of clinically relevant large animal models of acute hepatic failure, multi-center studies needed to accumulate experience in treating this process

Chronic Liver Failure from Cirrhosis

Clinically only anecdotal improvement in some liver functions reported

Barriers:

- Animal studies indicate hepatocyte transplantation may provide only short-term benefit
- Workable extrahepatic site for clinical hepatocyte transplantation not yet identified
- Fibrosis in the space of Disse limits engraftment of hepatocytes into the liver plates

Possible areas for progress:

- Additional laboratory research needed

The challenge of hepatocyte engraftment and treatment of liver-based metabolic deficiencies

In the long-term, transplanted hepatocytes appear to survive poorly in naïve normal and in immune deficient hosts [20,49,50]. They do, however, survive well in hosts with some forms of liver disease [51–53], and when native liver cell expansion is inhibited by exogenous interventions [54,55]. These observations suggest that some homeostatic mechanism controls the number of surviving donor hepatocytes over time. Graft survival, thus, could be limited by a host cell survival advantage over donor hepatocytes. This situation would be similar to that seen in allogeneic bone marrow transplantation, where the host must undergo a preparative regimen to create an environment conducive to long-term engraftment. Preparative irradiation induces apoptosis of host bone marrow cells and makes room for donor cell engraftment [56], allowing macrochimerism to take place following infusion of donor hematopoietic stem cells. Therefore, the use of hepatocytes for the treatment of liver-based metabolic disease may inevitably fail unless conditions can be established that will allow the enduring survival of hepatocyte transplants, as observed in some forms of liver damage.

Liver-directed radiation has been shown to facilitate repopulation of the native liver by transplanted hepatocytes when it is combined with a hepatic proliferation stimulus [57]. In fact, it has been shown that providing only the hepatic proliferation stimulus results in mild enhancement of hepatocyte engraftment for up to 16 weeks in non-human primates [58]. This is especially important since the number of donor cells that can be safely transplanted into the liver at any one time via the portal vein is small, usually less than 1% of the liver mass. Transplantation of a larger cell mass leads to either severe portal hypertension or translocation of cells out of the liver into the systemic circulation, leading to embolization of cells into the lungs [7,32]. Liver-directed radiation-based preparative regimens inhibit host hepatocyte proliferation and induce post-mitotic hepatocyte death, making "room" for donor hepatocytes to preferentially proliferate and repopulate the irradiated host liver [57,59]. This strategy has been employed to completely correct rodent models of hereditary metabolic deficiencies of the liver corresponding to Crigler–Najjar syndrome and primary hyperoxaluria [60,61]. Unfortunately, donor hepatocyte engraftment, survival, and repopulation studies greater than 2 years cannot be achieved in rodents, and pre-clinical studies in large animals would be helpful to confirm the safety and efficacy of such a strategy. For diseases that may require fairly limited replacement of the host liver by transplanted donor hepatocytes, reversible partial portal vein embolization may result in adequate donor cell function without the additional risks associated with conditioning the recipient liver with irradiation, although the long-term efficacy of this strategy has not yet been demonstrated (Fig. 1).

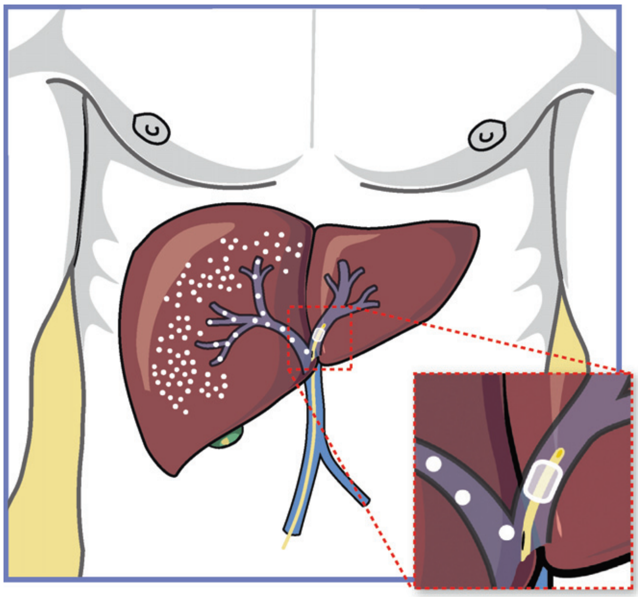

Fig. 1. Partial portal vein occlusion. Laboratory studies indicate that transient occlusion of the portal circulation can enhance donor hepatocyte engraftment by providing a proliferation signal to donor cells, with or without conditioning of the recipient liver by partial irradiation. Transient occlusion of the left portal venous system is shown, allowing transplantation into the right lobe of the liver. A 6 Fr compliant balloon is positioned in the left portal vein just beyond the bifurcation. It is inflated so as to occlude the left portal vein but allows transportal infusion of cells into the right lobe of the liver through the side port of the endovascular sheath.

The risk of modest doses of liver irradiation in infants using the above strategy should be low based on the experience in treating infants with symptomatic liver hemangiomas, and long-term follow-up studies in Wilm's tumor patients. From 1950s to 1980s, several reports were published concerning infants (approximately 20) treated with radiation therapy for symptomatic liver hemangiomas that demonstrated that radiation could be safely administered from a single dose of 7 Gy to fractionated doses of up to 50 Gy to portions of the liver. The age of these patients ranged from 1 day to 1 year. Thus, one-third of the normal liver volume may tolerate radiation doses as high as 80–100 Gy without compromising normal liver function. Furthermore, radiation-induced secondary liver cancer has not generally been reported in patients that receive radiation therapy involving external beam treatment [62–66]. In the National Wilm's Tumor Study, where 2438 patients were followed over 14,381 person-years, only four cases of hepatocellular carcinoma were reported in long-term follow-up, and this was only in patients who received radiation doses $\geqslant$35 Gy to the right lobe of the liver. Three out of four of those patients also received chemotherapy, which could have contributed to the second malignancies.

In Gunn rats, an animal model for the hyperbilirubinemia associated with human Crigler–Najjar syndrome type I, preconditioning with focused lobar irradiation to as little as 35% of the liver mass prior to allogeneic hepatocyte transplantation results in complete correction of the liver-based metabolic disorder [67]. Thus, for clinical application, it should be possible to radiate a portion of the liver in a range lower than the threshold to induce significant liver injury in order to augment engraftment and replacement of the host liver with donor hepatocytes. Since liver-directed radiation therapy can be safely administered in the clinic using 3-D conformal and intensity-modulated radiation therapy (IMRT) techniques, and can be easily adapted to selectively irradiate part of the liver or a liver lobe without collateral injury to surrounding structures, it should be possible to design safe and clinically effective strategies for irradiating the host liver, and engrafting and expanding donor hepatocytes there (Box 2).

Key points

Hepatocyte transplantation for metabolic liver disease

Partial correction of a number of metabolic liver disorders has been reported in patients

Barriers:

- Evidence of long-term engraftment and function by transplanted cells lacking

Possible areas for progress:

- In animals, complete correction of metabolic liver disease by hepatocyte transplant has been reported following radiation of the liver combined with a hepatic proliferation signal. Translation of this strategy to patients requires investigation.

The shortage of human donors

A major limitation to the clinical application of hepatocyte transplantation has been the lack of an abundant source of human hepatocytes. Hepatocytes are primarily obtained from livers rejected for orthotopic liver transplantation, and unused segments of donor livers used for pediatric recipients [68]. But, these sources do not begin to approach the potential numbers needed to treat all patients that might potentially benefit from hepatocyte transplantation.

One exciting new source of human hepatocytes may be livers from non-heart-beating donors. A recent study documented that these livers, of dubious quality for whole liver transplantation, can generate hepatocytes with acceptable viability and quality [69]. The primary limitation to utilizing these whole organs is the risk of long-term biliary and vascular complications, issues that are of little consequence when only hepatocyte yield and viability are critical.

The impact of the scarcity of livers might be alleviated, to some extent, by cryopreservation of isolated cells, allowing harvest and use of hepatocytes in the absence of an immediately available recipient. To this point, cryopreserved hepatocytes have not proven reliably capable of engrafting and functioning. Some children with urea cycle disorders have been treated with some possible success using cryopreserved hepatocytes [70]; however, the quality of lots of cryopreserved cells and their ability to engraft after thawing has not been shown to be consistent by others [71–76].

Hepatocytes might also be obtained by expansion and differentiation of stem cells. While this possibility has generated enthusiasm, it remains some distance from application. Embryonic stem cells and induced pluripotent stem cells can be coaxed to exhibit some functions of hepatocytes by sequential culture in transcription and growth factors, and sorting to enrich for cells with hepatocyte-specific characteristics [77,78]. This is difficult to accomplish in large numbers and there is still a significant risk of contamination of the enriched population with cells having the potential to form tumors.

The ideal source of stem cells would be derived from the subject to be treated. Such stem cells, referred to as induced pluripotent stem cells (iPS cells), have been created by transducing skin cells, or any of a number of other differentiated cell sources, with transcription factors that transform them into cells that have characteristics and differentiation potential nearly identical to human embryonic stem cells (ES cells) [79,80]. While there is much enthusiasm for the potential use of stem cell-derived hepatocytes, generation of a sufficient mass of functional hepatocytes for treatment of liver failure from autologous cells derived from iPS cells would require a period of weeks for expansion, differentiation, selection, and testing to exclude contamination by tumorogenic precursors, far too long to address the problem of acute hepatic failure. In addition, autologous hepatocytes would require genetic manipulation to treat a metabolic disease and such manipulation might result in changes that could increase cancer risk. Thus, numerous hurdles and unresolved risks make this source of hepatocytes unlikely to be useful for clinical transplantation in the near future.

Finally, xenotransplantation of hepatocytes could address many of the challenges of treating liver disorders. It is not limited by the availability of donors, could be performed repeatedly if needed, and may be more effective than allotransplantation for the treatment of viral hepatitis, since xenogeneic hepatocytes do not appear susceptible to infection by human hepatitis viruses [81,82]. Hepatocyte xenotransplantation has been performed with some success in small animals with hypercholesterolemia [83]. In addition, porcine hepatocytes have been shown to secrete albumin for periods of months in naïve non-human primates [49], where the enduring survival and function of the grafts was achieved using a "conventional" immune suppression regimen, and did not require the use of donor pigs genetically engineered with disrupted synthesis of Galα1-3Gal, or expressing proteins that inhibit activation of complement by bound antibodies [84–90]. Importantly, rats transplanted with hepatocyte xenografts had improved indices of coagulation, less encephalopathy, and survived longer than cirrhotic rats that did not receive hepatocyte xenografts [42], and the porcine hepatocytes engrafted and corrected liver failure for nearly 2 months without the need for immune suppression. The hepatocyte xenografts caused the rodent recipients in liver failure to be sensitized, but the immune response did not damage already engrafted cells. Thus, the need for immune suppression following hepatocyte transplantation in liver failure could be extremely low. Development of a hepatocyte xenotransplantation program in patients would need to be initiated with caution, as the possibility of transferring an infection across species from the donor animal to man could result in a significant public health concern.

Unfortunately, in the absence of clinically relevant models for human liver disease in non-human primates [91,92], it has so far not been possible to predict the extent to which a hepatocyte xenograft would restore hepatic function in humans. Baboons transplanted with livers derived from transgenic pigs expressing the human complement decay accelerating factor supported the recipient's life for eight days, and clotting parameters reached nearly normal levels within two days of transplantation [93]. Thus, since transplantation of isolated hepatocytes into the liver is much less invasive than whole liver transplantation and the immunologic barrier appears lower, hepatocyte xenotransplantation could become the preferred treatment for conditions in which some liver function persists or can recover, such as in fulminant liver failure, when immediate availability of donor hepatocytes could be a decisive factor in the patient's outcome.

Summary

Treatment of patients with liver disease by hepatocyte transplantation has expanded dramatically over the last decade, especially for treatment of patients with liver-based metabolic disorders. While much progress has been made, full realization of its potential has not been reached. Treatment of acute liver failure has been hampered by a number of factors, but the efficacy of hepatocyte transplantation in treating this entity could be better determined through a multi-institutional trial using a uniform and standardized treatment strategy. The barriers to treating chronic liver failure resulting from cirrhosis are more extensive. Novel strategies are being developed to safely precondition patients with liver-directed radiation therapy in order to enhance donor hepatocyte survival, long-term engraftment and improve treatment of patients with life-threatening liver-based genetic deficiencies. This work could soon be translated to the clinic. Once hepatocyte transplantation has been shown to effectively replace organ transplantation for a portion of patients with liver failure and life-threatening liver metabolic diseases, it is likely that multiple novel sources of donor hepatocytes will be developed, making cell therapy available and effective for a broad population of patients with liver disorders.

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