REVIEW ARTICLE

Hepatocyte Transplantation

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ABSTRACT

Orthotopic liver transplantation is an established treatment for patients with severe acute and end-stage chronic liver disease. The shortage of donor organs continues to be the rate-limiting factor for liver transplantation throughout the world. Hepatocyte transplantation is a promising treatment for several liver diseases and can, also, be used as a 'bridge' to liver transplantation in cases of liver failure.

Keywords: Hepatocytes, Isolation, Transplantation, Cryopreservation.

INTRODUCTION

Liver transplantation is the gold standard treatment for end-stage liver failure and numerous liver-based inborn errors of metabolism.[1]

The shortage of organ donors is a problem worldwide, with approximately 15% of adult patients with life-threatening liver diseases dying while on the waiting list.[2]

Presently, cell therapies represent one of the most promising alternative solutions to entire or partial liver transplantation.[3] The use of autologous cells overcomes the problems associated with donor scarcity and immuno-suppression.[4]

Hepatocyte transplantation (HT) is a promising treatment for several liver diseases and can be used as a 'bridge' to liver transplantation in cases of liver failure.[5]

The first human trial of HT was not achieved until 1992 when Mito et al performed a partial hepatectomy on 10 patients with chronic cirrhotic liver failure. The recipients' native cirrhotic left lateral liver segments were used as the cell source for HT and there was improved encephalopathy in two cases.[6]

As of 2006, only 78 hepatocyte transplants had been performed worldwide. Of those transplants, 21 were delivered to patients suffering from inborn errors of metabolism, 20 patients suffering from chronic liver diseases and the remaining 37 transplants were delivered to patients with acute liver failure.[7]

Most of the available studies focus on adult HT and the results are neither reproducible nor comparable, because the means of infusion, amount of injected cells and clinical variability differ among various studies.[2]

The efficacy of HT varies according to the etiology of the disease and remains principally dependent on the quality of the initial liver cell suspension used.[8]

DISCUSSION

Isolation and Infusion Techniques

The isolation of hepatocytes must meet the standards of good manufacturing practice. The liver is digested by collagenase perfusion with modifications of the Seglen technique and variations in the buffer composition,[4] and the hepatocytes obtained are generally transplanted fresh or thawed after cryopreservation.[8]

Rodent models as well as pilot trials using allogeneic hepatocytes to treat a type 1 Crigler-Najjar metabolic disease have revealed the benefits of using freshly isolated and uncultured hepatocytes.[9]

The liver and the spleen are the most reliable sites used for transplantation.[2]

In cases of metabolic liver disease and acute liver failure in which the liver architecture is intact, the presence of physiological matrix and the availability of portal blood supply make the liver the optimal site for HT.[4]

The spleen has proven to be the best site for hepatocyte engraftment.[10] Hepatocytes infused into the portal vein or infused directly into the spleen undergo blood flow–mediated translocation to the hepatic sinusoids.[11] However, a large proportion remains in the spleen, which can serve as a site for long-term survival and function of engrafted hepatocytes.[2]

Intrasplenic HT has been performed in animal models of chronic liver failure. In rats, 2×107 hepatocytes (representing approximately 3% of the host liver mass) to 7.5×107 hepatocytes (approximately 12.5% of the host liver mass) were transplanted into the splenic parenchyma, and only a transient portal hypertension was observed.[4]

Hepatocytes can function in ectopic sites, such as the peritoneal cavity with some clinical effectiveness.[2] Clinical and biochemical improvements have been reported in several studies of humans with fulminant hepatic failure (FHF) treated with intraperitoneal HT.[12] Unfortunately, these sites do not efficiently support cell viability for long periods because the transplanted cells have no direct and instant access to oxygen and nutrients.[4] However, the survival of hepatocytes transplanted into the peritoneal

cavity can be prolonged by encapsulation in alginate or attachment to collagen-coated beads.[13]

The renal capsular space has, also, been used, but it can accommodate only a small number of liver cells.[4]

Cell Death

Cell death of freshly isolated hepatocytes occurs by apoptosis.[14] Apoptosis is initiated when hepatocytes are isolated by separation of anchorage-dependent hepatocytes from their extracellular matrix components, a process called anoikis or homelessness.[15] This process appears to involve caspase activation following cell-matrix detachment.[4,16]

Encapsulation

Hepatocytes have been encapsulated prior to cryo-preservation to confer mechanical protection.[8]

Encapsulated cryopreserved rat hepatocytes injected intraperitoneally into a mouse model of fulminant liver failure remained viable up to 2 weeks post-transplantation.[17]

Various methods of bioencapsulation have been developed to maintain the specific functions and phenotype of the cells.[4] They include supplementation of factors, use of appropriate substrates such as alginate, and cocultivation of hepatocytes with other types of cells. For example, hepatocytes have been encapsulated with bone marrow cells including stem cells.[18] Syngeneic bioencapsulated bone marrow cells transdifferentiate into hepatocyte-like cells in a rat model of acute injury.[19]

Techniques to microencapsulate hepatocytes within synthetic semipermeable membranes have been developed to enable the transplantation of cell cultures without the need for immunosuppression.[4] Hepatocytes can be microencapsulated within a collagen matrix enveloped in an ultrathin sodium alginate copolymer membrane, which allows molecules such as glucose, albumin and clotting factors to diffuse freely but prevent the microencapsulated cells from getting out.[20]

Encapsulated hepatocytes have been applied in the treatment of liver failure either after intraperitoneal or intrasplenic transplantation into rats.[4]

Cryopreservation

Cryopreserved cells are, also, used in experimental and clinical trials using allogeneic hepatocytes.[4,21] The functionality of which, however, remains lower than that of freshly isolated hepatocytes.[22]

Hepatocytes are highly susceptible to the freeze-thaw process.[4] Various factors affect the viability and function of thawed cells. They include the preincubation of hepatocytes with cytoprotective compounds to promote

recovery from the isolation process prior to cryopreservation, the freezing solution and the freezing protocol.[8]

There are differences in the resistance of hepatocytes to cryopreservation according to the species and the developmental age.[4] For example, preincubation with fructose improves the viability and attachment efficiency of rat hepatocytes and improves the attachment efficiency of human hepatocytes.[21]

The addition of trehalose or other related oligo-saccharides to the cryopreservation medium improves the post-thaw cell viability and plating efficiency of both rat and human hepatocytes.[23] Trehalose is a naturally occurring (though not found in the human body) disaccharide containing two glucose molecules. This saccharide was tested because lower organisms such as fungi, yeast, bacteria and insects, which have the ability to survive complete freezing and/or drying, accumulate a large amount of trehalose.[24]

Immortalization

Transplantation of animal models with immortalized hepatocytes is based on the hypothesis that an ideal alternative to the transplantation of primary human hepatocytes would be the use of a clonal cell line that could be grown in culture and exhibit the characteristics of differentiated nontransformed hepatocytes following transplantation.[25]

In 1996, Westerman and Leboulch described an appropriate approach which is called reversible immortalization. Reversible immortalization was achieved by immortalization of rodent cells with a retroviral vector expressing the Simian virus 40 large T antigen (SV40Tag) gene and a suicide gene, Herpes simplex virus thymidine kinase flanked by a pair of loxP recombination targets.[4] This approach allows hepatocytes expansion *in vitro*.[26] Moreover, immortalized hepatocyte clones when transplanted into the spleens of rats with liver failure, prevented the development of hyperammonemia-induced hepatic encephalopathy.[4] However, transplantation of immortalized cells cannot currently be envisaged in clinical settings.[4]

Liver Conditioning

It has been recently shown that liver conditioning by irradiation causes prolonged cell cycle block and promotes preferential proliferation of transplanted hepatocytes.[27] However, this procedure and others used in rodents, including hepatectomy and injection of toxins, cannot be used in patients: Such liver conditioning carries unacceptable clinical risks.[28]

In rats, portal branch ligation, i.e. the occlusion of portal branches of the two anterior liver lobes (70% of the total liver mass) results in a regenerative response in the remaining nonoccluded lobes leading to their hypertrophy.[29] A similar approach was performed in Watanabe hyperlipidemic rabbits.[4] Five months after the transplantation of hepatocytes into portal branch ligation-stimulated Watanabe rabbits, the decrease in cholesterol was more pronounced and sustained than that in nonligated animals.[30]

Portal branch ligation, also, favors efficient retroviral transduction of hepatocytes *in vivo*.[31]

Applications of HT

Animal Models

More than 30 years after the first HT to treat the Gunn rat, the animal model for Crigler-Najjar syndrome, with marked reduction of serum bilirubin, there are still a number of impediments to HT.[9]

Numerous animal models are used in work aimed at complete elucidation of the molecular mechanisms of hepatocyte engraftment, improving engraftment process and/or long-term function.[4]

Small animal models able to harbor functional human liver cell xenografts have been developed using immunodeficient mice.[32] Successful transplantation of human hepatocytes into mice or rats requires the recipient animals to provide a supportive niche that promotes engraftment of the cells.[4]

Research with large animal models (rabbits, pigs, dogs or nonhuman primates) is, also, essential to define procedures that can be applied clinically.[33] Various important issues also need to be addressed in such animal models, including how many cells can be transplanted safely either once or repeatedly and the numbers of cells needed to achieve therapeutic goals.[34]

The rabbit is a lagomorph and considered to be a large animal model, however, rabbits are very sensitive to stress and anesthesia, and they, also, are prone to developing portal thrombosis after cell transplantation even if given high doses of heparin.[4]

Pigs are suitable for the assessment of HT and bioartificial livers and have been recently reported to be a reliable model for liver failure.[34]

Clinical Trials

Chronic liver diseases: The first human hepatocyte autotransplants were performed in 1992 in 10 patients with chronic liver disease, using the left lateral segment as the cell source (Table 1).[35] Transplanted hepatocytes were detected in the spleen with Tc99m labeling at 1 and 6 months.[2]

The next 10 patients were treated mostly with intrasplenic artery infusion of allogenic hepatocytes (in two cases, the infusion was intraportal) and six had some improvement in encephalopathy, hepatic protein synthesis and renal function. A liver transplant recipient with acute graft dysfunction, who had received an intraportal infusion, developed portal thrombosis and died the same day, while, the remaining three patients died later on (Table 1).[12]

Fulminant hepatic failure: Patients with FHF have the highest mortality while on the waiting list, with an estimated 10% survival. HT can potentially support liver function until an organ becomes available or the liver regenerates.[2]

In a study done in 1994, fetal hepatocytes (6×10^7/kg body weight) were injected in 10 patients intraperitoneally through a dialysis catheter. Three of them recovered, showing neurological improvement and decreased ammonia and bilirubin levels just 48 hours after the infusion.[36]

Among the 22 patients with FHF who received adult HT (Table 1),[11] had splenic artery infusion, nine had portal vein infusion and two received both splenic and intraportal infusion.[38] Nine patients had a complete recovery (seven of whom received LT). Two patients with herpes simplex virus and one with hepatitis B virus-related liver disease died.[38]

Inborn errors of metabolism: The concept of liver cell transplantation (LCT) as a new therapeutic concept for inborn errors of metabolism was introduced into clinical practice in 1994, 2 years after the first application in human patients with liver cirrhosis.[35]

In 1994, Grossman et al treated five patients with homozygous familial hypercholesterolemia by transplantation of genetically modified autologous hepatocytes.[39] Three years later, the first LCT for metabolic disease with allogeneic donor cells was performed in a 5-year-old boy with ornithine transcarbamylase (OTC) deficiency.[40] Since then, about 27 children with hepatic-based metabolic diseases have undergone allogeneic LCT, through portal or umbilical vein, with encouraging results.[41]

Among children with urea cycle disorders, three patients had NH_3 control and evidence of OTC activity on liver biopsy.[2] A 3.5-year-old girl with argininosuccinate lyase (ASL) deficiency and psychomotor retardation received a total of 4.7×10^9 hepatocytes (divided into 11 infusions), with important ammonium level reduction, a 3% ASL activity on liver biopsy at 8 months (undetectable at baseline), and evidence of engrafted male cells at 1 year.[42]

A 9-year-old Crigler-Najjar type 1 child achieved a 50% reduction of bilirubin after receiving 5% of the hepatic mass divided into three intrahepatic infusions over 24 hours.[43]

Table 1: Adult hepatocytes transplantation in chronic liver disease and FHF[2]				
	Patients	*Viable cell range*	*Outcome Died/Alive/LT*	*References*
Chronic liver disease	20	1.7×10^7		
Autotransplant	10	6.0×10^8	–	35
Allotransplant	10		4/6/3	
• Alcohol	5	Not available	2/3/-	50
• $\alpha 1$ antitrypsin deficiency	1	2.2×10^7	/1/1	40
• HCV-related	1	7.5×10^6	1/-/-	40
• Others	3[1]	5.0×10^8-2.0×10^9	1/2/2	13
Fulminant hepatic failure	22		13/9/7	
Viral (HSV and HBV)	6	1.2×10^8-3.0×10^{10}	3/3/2	50
				40
Drug-related	10	2.8×10^7-3.9×10^{10}	8/2/2	40,37
Idiopathic	4[2]	1.8×10^8-4.0×10^9	1/3/3	50
Others	2	1.7×10^8-4.9×10^8	1/1/-	38

LT: Liver transplantation; HCV: Hepatitis C virus; HSV: Herpes simplex virus; HBV: Hepatitis B virus; [1]Cryptogenic cirrhosis (n = 1), idiopathic fibrosis (n = 1) and liver transplant recipient (n = 1); [2]Mushroom poisoning (n = 1) and trisegmentectomy (n = 1).

Hereditary diseases: Three children with factor VII deficiency showed 80% reduction in exogenous factor VII replacement up to 6 months after transplantation.[44]

Five patients with homozygous familial hypercholesterolemia were transplanted with autologous (left lateral liver segment resected) genetically modified hepatocytes, with an *ex vivo* transduced low-density lipoprotein (LDL) receptor gene. In three of them, a >20% reduction in LDL cholesterol was observed up to 28 months after liver-cell transplantation (the longest sustained reduction rate reported in pediatric cases).[2] Intraportal HT had no benefit for two children with progressive familial intrahepatic cholestasis, but the failure was attributed to significant liver fibrosis found at the time of LT.[44]

Fate of Engrafted Hepatocytes

Many studies have shown that hepatocytes transplanted into rodents via the spleen or the portal vasculature enter through portal vein branches and are entrapped in proximal hepatic sinusoids, which are 6 to 9 µm in diameter, i.e. they are distributed predominantly in periportal regions of the hepatic lobules.[4]

Numerous hepatocytes (up to 70% of transplanted cells) remain trapped in the portal spaces, and most of them are destroyed by the phagocytic responses of Kupffer cells, which are activated shortly after deposition of hepatocytes in liver sinusoids.[45] The remaining cells translocate from sinusoids into the liver plates through a process involving disruption of the sinusoidal endothelium and release of vascular endothelial growth factor by both host and transplanted cells.[46] Translocated cells integrate into the liver parenchyma, where gap junctions and bile canaliculi form between transplanted and host hepatocytes without any significant proliferation in adult animals.[47]

Transplantation of 2×10^7 hepatocytes in rats has led to the engraftment of about 0.5% of the transplanted cells in the recipient livers.[48] Only hepatocytes harboring a selective advantage for survival/proliferation can efficiently repopulate a recipient liver.[49]

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