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TOPIC HIGHLIGHT

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Clinical applications of hepatocyte transplantation

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Abstract

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. The use of cell transplantation for liver disease is an attempt to correct metabolic defects, or to support liver function as a bridge to liver transplantation and, as such, has raised a number of expectations. Most of the available studies briefly reported here focus on adult hepatocyte transplantation (HT), and the results are neither reproducible nor comparable, because the means of infusion, amount of injected cells and clinical variability differ among the studies. To better understand the specific role of HT in the management of end-stage liver disease, it is important that controlled studies, designed on the principles of evidence-based medicine, be done in order to guarantee the reproducibility of results.

Key words: Hepatocyte transplantation; Metabolic diseases; Chronic liver disease; Liver failure; Stem cells

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INTRODUCTION

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. Hepatocyte transplantation (HT) may therefore become a viable alternative treatment to liver transplantation (LTx) for these patients. From 1992 to date, several studies on adult human HT have been conducted in patients with acute or chronic liver failure, in an attempt to correct metabolic defects or support liver function as a bridge to LTx[1]. Hepatocytes are isolated from the patient's liver (autologous) or from discarded transplant organs (homologous). Other potential sources are livers obtained from non-heart-beating donors, marginal grafts (steatotic, liver trauma), and segment Ⅳ (with or without caudate lobe) from split-liver techniques, in which one liver is used for two recipients[2].

ISOLATION AND INFUSION TECHNIQUES

The isolation of hepatocytes must meet the standards of good manufacturing practice. The liver is digested by collagenase and the hepatocytes obtained are generally transplanted fresh or thawed after cryopreservation. Both types of cells seem to be efficient, although there is perhaps an advantage to using fresh cells. Although the liver and the spleen are the most reliable sites used, the peritoneal cavity has also been used for transplantation in patients with fulminant hepatic failure[3]. While the infusion route used for cell transplantation is usually the portal vein, the splenic artery or a direct splenic puncture have also been used. The choice of the organ to infuse should be based on the underlying liver architecture, which, in the case of cirrhosis, may limit the hepatocyte engraftment because: (1) there is diffuse and abundant extracellular matrix, i.e. a potential endothelial barrier for nesting; (2) the portosystemic shunts could favor the translocation of hepatocytes to the pulmonary circulation; and (3) the presence of portal hypertension may expose patients to the risk of portal thrombosis,

with the consequence of further deterioration of the existing liver function. Injection through the portal vein should then be reserved for correcting inborn metabolic errors, while the splenic artery should be considered for patients with a fibrotic liver. The splenic puncture poses too many risks for patients with splenomegaly and portal hypertension. For hepatocyte transplantation into the liver, up to 10^9 cells per treatment can been infused *via* the portal vein, either through an indwelling catheter into a branch of the inferior mesenteric vein or through a catheter placed transhepatically under radiographic control. During the infusion, it is essential to monitor the portal venous pressure to avoid the risk of portal hypertension. The hypothetical aim is to perform repeated cell infusions in order to provide approximately 5%-10% of total liver mass, though it is still not clear what constitutes the maximum number of liver cells that can be infused each time, how many infusions can be performed in total and what the required hepatocyte mass is, depending on the specific metabolic deficit and stage of chronic liver disease.

Transplanted hepatocytes engraft, proliferate and function metabolically, as shown by several animal models. Moreover, in humans, their capacity to engraft in the liver has been demonstrated in a female patient with acute liver failure who received 2.8×10^7 male hepatocytes through the splenic artery. Nested PCR for the Y chromosome was performed in the explanted liver 10 d after the infusion, showing an engraftment ratio of 1:4000[4]. The immunosuppression scheme resembles that used in whole-organ transplant, and is generally based on tacrolimus ± steroids.

CLINICAL STUDIES

Adult HT for metabolic liver diseases

Inborn errors of metabolism affect around 1 in 900 live births, and LTx is an accepted and successful treatment for liver-based metabolic disorders, with more than 90% of children achieving long-term survival[5]. The success of auxiliary LTx in humans[6] supports the observation in animal experiments that a relatively small amount of liver tissue can provide sufficient function to correct the underlying metabolic defects. The number of transplanted cells is between 5% and 10% of the liver mass, with a varying amount of cells depending on the use of fresh *vs* cryopreserved cells. About 27 children have received liver cell transplantation, through portal or umbilical vein, for inborn errors of metabolism. Among children with urea cycle disorders, three of them with ornithine transcarbamylase deficiency (OCT) had NH3 control and evidence of OCT activity on liver biopsy. 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 mo (undetectable at baseline), and evidence of engrafted male cells (12.5%) at 1 year[2,7-10]. A 9-year-old Crigler-Najjar type 1 baby achieved a 50% reduction of bilirubin after receiving 5% of the hepatic mass divided into three intrahepatic infusions over 24 h, and returned toward pre-transplant levels 2 years later, despite evidence of functioning, engrafted allogenic hepatocytes[12]. 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 more than 20% reduction in LDL-cholesterol was observed up to 28 mo after liver-cell transplantation (the longest sustained reduction rate reported in pediatric cases), but with evidence of a < 5% transgene expression at 4 mo[15]. Three children with factor Ⅶ deficiency showed an 80% reduction in exogenous factor Ⅶ replacement up to 6 mo after transplantation[11,16]. 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 LTx[11]. Twelve patients, who had received HT as a bridge to transplantation, subsequently underwent elective orthotopic LTx (Table 1).

Table 1 Adult HT in metabolic liver disorders

	Patients (n = 27)	Range of viable[1] cells number	Outcome (died/LT)	References
Urea cycle (OTC/ASL/ASS: 5/1/1)	7	$1.9\text{-}4 \times 10^9$	1/4	[2,7-10]
Crigler-Najjar type 1	6	$1.5\text{-}7.5 \times 10^9$	-/3	[11-14]
Hyper-cholesterolemia	5	$1.0\text{-}3.2 \times 10^9$	-/-	[15]
Factor Ⅶ deficiency	3	$1.1\text{-}2.2 \times 10^9$	-/2	[11,16]
Others	6[2]	$3.2\text{-}7.5 \times 10^9$	-/3	[11,17,18,20]

[1]In a few cases several cell infusions were performed; in one patient up to 18 infusions; [2]Glycogenosis type 1 (n = 2); refsum disease (n = 1); progressive familial intra-hepatic cholestasis (n = 2); α-1-antitrypsine deficiency (n = 1).

Adult HT for chronic liver disease and fulminant hepatic failure

Twenty patients have received HT for chronic liver disease. The first human hepatocytes were autotransplants performed in 1992 in 10 patients with chronic liver disease, using the left lateral segment as cellular source[19]. Transplanted hepatocytes were detected in the spleen with Tc 99m labeling at 1 and 6 mo. The next 10 patients were treated mostly with intrasplenic artery infusion (in two cases, the infusion was intraportal) and had some improvement in encephalopathy, hepatic protein synthesis and renal function. Four of them died. A liver transplant recipient with acute graft dysfunction, who had received an intraportal infusion, developed portal thrombosis and died the same day (Table 2)[4,20-22].

Patients with fulminant hepatic failure (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. In a 1994 study[3], fetal hepatocytes

Table 2 Adult hepatocyte transplantation in chronic and fulminant hepatic failure

	Patients	Viable cells range	Outcome died/alive/LT	References
Chronic liver diseases	20			
Autotransplant	10	1.7×10^7-6.0×10^8	/	[19]
Allotransplant	10		4/6/3	
Alcohol	5	/	2/3/-	[20]
α-1-antitrypsine deficiency	1	2.2×10^7	-/1/1	[4]
HCV related	1	7.5×10^6	1/-/-	[4]
Other	3[1]	5×10^8-2×10^9	1/2/2	[21,22]
FHF	22		13/9/7	
Viral (HSV, HBV)	6	1.2×10^8-3×10^{10}	3/3/2	[4,20,23]
Drug-related	10	2.8×10^7-3.9×10^{10}	8/2/2	[4,13,20,22,23]
Idiopathic	4	1.8×10^8-4×10^9	1/3/3	[20,22]
Other	2[2]	1.7×10^8-4.9×10^8	1/1/-	[1,20]

[1]Cryptogenic cirrhosis (n = 1); idiopathic fibrosis (n = 1); liver transplant recipient (n = 1); [2]Mushroom poisoning (n = 1); trisegmentectomy (n = 1).

(60×10^6/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 h after the infusion. No complications were related to the procedure. Among the 22 patients who received adult HT (Table 2), 11 had splenic artery infusion, nine had portal vein infusion and two received both splenic and intraportal infusion. Nine patients had a complete recovery (seven of whom received LTx). Two patients with herpes simplex virus and one with hepatitis B virus-related liver disease died[1,4,13,20,22,23].

FUTURE PERSPECTIVES

Most of the studies done in this field still focus on adult hepatocytes for transplantation, because this type of hepatocyte is considered a potential resource for bridging to LTx. However, this emphasis should perhaps be tempered by two important considerations: (1) adult hepatocytes are scarcely available, since they are obtained principally from discarded organs that cannot be transplanted; and (2) adult hepatocytes have limited proliferative capacity.

Alternative cell sources with vast capacities to consider for clinical application are stem cells and stem-cell-derived hepatocytes. Fetal tissues are in fact already deemed by the scientific community to be a promising source of liver stem cells to be used for clinical purposes. In Europe (Italy included), a multicenter study is underway on the use of fetal neuronal cells for the treatment of degenerative diseases. A study published in 2000 showed a functional improvement of cognitive-motor abilities in patients with Huntington's disease after human fetal neuron transplantation[24]. Fetal liver cells have several advantages compared to adult liver cells: greater availability, proliferative capacity and plasticity, less immunogenicity, good adaptation and integration capacity, and greater resistance to cryopreservation and ischemia. Moreover, there are no reports of oncogenic transformation, at least 2 years after intrasplenic fetal hepatocyte transplantation, in animal models[25].

The use of cell transplantation for liver disease raises a number of expectations, though it is important that controlled studies designed on the principles of evidence-based medicine be done in order to guarantee the reproducibility of results. At the same time, before establishing the safest and most effective number of cells to be infused, an accurate method for quantifying the engraftment rate, associated with specific tests of hepatocyte functionality, should be developed. A strict selection of candidates, and stratification by clinical scores (e.g. Meld score), could finally help clinicians to better understand the specific role of HT in the management of end-stage liver disease.

CONCLUSION

The results available in the literature are neither reproducible nor comparable. The means of infusion, the amount of injected cells and the clinical variability differ among the studies. In addition, a well-defined protocol of clinical and biochemical monitoring has yet to be established. However, the partial correction of the inborn errors, the sustained engraftment of at least 1/8 of the infused hepatocytes, and the prolonged survival in pediatric patients with metabolic liver diseases are encouraging enough to consider adult HT an effective bridging procedure to LTx for this category of patients. As it can be seen from the cases summarized in Table 2, 40.9% of patients with FHF and 60% of patients with chronic liver disease benefited from the hepatocyte infusion because they survived, with or without LTx. Nevertheless, it is possible that patients who recovered could have done so by spontaneous remission of the disease. Otherwise, in the remaining half of the patients, it could be hypothesized that the reason HT was not effective was attributable to the paucity of cells injected (rather than the loss of hepatic function in liver failure), or to the immunosuppressive regimens used (based on those for whole organ transplantation), which were not optimal for guaranteeing the function of the transplanted cells.

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