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Hepatocyte Transplantation in Special Populations: Clinical Use in Children

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Abstract

Orthotopic liver transplantation remains the only proven cure for end-stage liver failure. Despite significant advances in the field, the clinical demand for donor organs far outweighs the supply. Hepatocyte transplantation has been proposed as an alternative approach to whole liver transplant in select diseases. Several international centers have reported experimental trials of human hepatocyte transplantation in acute liver failure and liver-based metabolic disorders. This chapter provides an introduction to hepatocyte transplantation from both a technical and clinical perspective. We will also focus on the special needs of pediatric patients, since historically the majority of clinical hepatocyte transplants have involved infants and children.

Keywords

Cell therapy; Human hepatocytes; Hepatocyte transplant; Metabolic liver disease; Pediatric liver disease; Regenerative medicine; Gene therapy

1 Introduction

The first report of orthotopic liver transplantation in humans was published by Dr. Thomas Starzl over 50 years ago [1]. One of the three patients described was a 3-year-old boy with biliary atresia who received preparative immune suppression followed by transplantation of a deceased donor liver from a 3-year old who succumbed to a brain tumor. Unfortunately, the pediatric recipient died of exsanguination on the operating table, 4 h after revascularization of the homograft. In the decades since that momentous pediatric liver transplant, the procedure has been refined considerably in terms of surgical techniques, immunosuppressive regimens, and postoperative management, leading to improved clinical outcomes with long-term patient and graft survival. Once liver transplantation was established as the primary curative treatment for acute and chronic liver failure, its clinical application was soon expanded to include a variety of liver-based metabolic diseases, generally comprised of inherited single enzyme defects diagnosed shortly after birth. Inborn errors of metabolism are individually rare but collectively common. As a result, the number of pediatric patients awaiting liver transplantation has significantly increased, with more diverse etiologies of liver disease compared to those usually seen in adults (e.g., chronic hepatitis C, alcoholism, acetaminophen toxicity). Since the waiting list for transplantable whole organs far outweighs the supply, liver cell transplantation and other innovative strategies have been proposed to expand the donor pool. Recent studies using auxiliary partial liver transplantation as a bridge for native liver support is proof of principle that achieving a critical mass of donor hepatocytes can be sufficient to restore parenchymal

defects. Although cell therapy for liver disease is still considered experimental, cumulative results from a number of international centers have shown potential for clinical hepatocyte transplantation in children, particularly for acute liver failure and liver-based metabolic diseases.

2 Clinical Hepatocyte Transplantation in Children

2.1 Special Needs of the Pediatric Liver Patient

Treatment of pediatric liver diseases has long been associated with the evolution of clinical hepatocyte transplantation. Of the over 140 published cases of human hepatocyte transplantation, the vast majority have been performed in children (summarized in Tables 1 and 2). It is therefore necessary to examine the unique needs of the pediatric liver transplant candidate, and how these may be fulfilled by hepatocyte transplantation. In general, children under 12 years old are assessed with specialized listing criteria, known as the Pediatric End-stage Liver Disease (PELD) scoring system [2]. Compared to the Model for End-stage Liver Disease (MELD) scoring system used in adults, both systems assess the degree of liver dysfunction; however, the PELD score also incorporates a child's age and extent of growth failure at the time of listing, underscoring the nutritional and developmental morbidity of pediatric liver diseases. In addition to these weighted parameters, certain liver-based metabolic diseases, where parenchymal function is largely preserved, also qualify for exception points to offset normal liver indices. More recent modifications to orthotopic liver transplantation, such as grafts obtained from split livers and living donors, have expanded the donor pool for children. Taken together, the aforementioned factors offer children more opportunities for successful clinical outcomes; however, they also reflect the growing necessity to develop alternatives to liver transplantation.

2.2 Advantages of Hepatocyte Transplantation in Children

Theoretically, clinical hepatocyte transplantation offers several advantages to children awaiting liver transplantation. Cell infusions are by far safer, less invasive, and more cost effective than transplanting a whole organ. Depending on the recipient, the goal of hepatocyte transplantation can be supportive or curative. In either case, a recipient can benefit from the engrafted donor cells, while still maintaining the native liver. Specific indications for liver cell therapy in children include acute liver failure (Table 1) and metabolic liver diseases (Table 2). Allogeneic hepatocyte transplantation for acute liver failure has been used as a “bridge” therapy, similar to auxiliary liver grafts, to support the failing liver and allow time for the patient either to recover or to receive an organ transplant [3]. In the former scenario, a foreseeable endpoint is adequate regeneration of the native liver rather than permanent engraftment of transplanted cells, limiting the need for lifelong immunosuppression and its side effects. In theory, antirejection medications could be safely withdrawn once native liver function has returned, leading to iatrogenic graft loss. Clearly, two major benefits hepatocyte transplantation offers to children are reduced immune suppression and preservation of the native liver.

In contrast to pediatric acute liver failure, a variety of approaches for both allogeneic and autologous hepatocyte transplantation have been described in patients with liver-based

metabolic diseases (Table 2). Children with such disorders typically require lifelong specialized diets to prevent toxic metabolites, but still run the risk of breakthrough metabolic crises from acute illness or poor compliance, leading to irreversible neurologic sequelae. Early studies estimated that only a small number of engrafted cells, representing ~5 % of the native liver mass, is sufficient to reconstitute a single enzyme defect, potentially offering these patients dietary liberalization, less neurodevelopmental impairment, and improved quality of life, even if partially corrected [4, 5]. Unlike organ transplantation, loss of engrafted cells in this population would not require emergent retransplant, since the patient could simply resume the pretransplant diet with an intact native liver. Determining the critical mass required for long-term correction of a particular condition may be achieved by planned sequential hepatocyte transplantation of fresh or cryopreserved cells, with close monitoring of metabolites. Theoretically, these cells could also provide auxiliary support to liver transplant, as wait times can be longer in metabolic patients. Liver cell therapy may even be curative in the setting of targeted gene correction in autologous cells (e.g., iPS-derived hepatocytes). Although uniform protocols are lacking, autologous cell transplantation can potentially eliminate lifelong immunosuppressant dosing and its side effects in children.

2.3 Disadvantages of Hepatocyte Transplantation in Children

Despite such perceived and actual benefits, human hepatocyte transplantation is still far from routine clinical practice. Although partial improvement in disease severity has been achieved, long-in Children term cures have not been demonstrated with liver cell transplantation. Regarding acute liver failure, over 40 total patients have been treated worldwide, but no survival benefit was achieved despite improvements in liver biochemistries and hepatic encephalopathy [6]. Similarly, over 30 total patients treated for urea cycle defects showed initial clinical improvement; however, donor cell function generally declined after 9–12 months [6]. As most pediatric metabolic diseases are diagnosed in infancy and treated with cell infusions at a young age, the expectation for lifelong graft survival in children may be unrealistic.

As discussed in a 2009 international consensus meeting, a major hindrance to suboptimal clinical outcomes has been a lack of standardization and controlled clinical trials [7]. Since the first reports of human hepatocyte transplantation in the 1990s, 18 different groups from around the globe have published case series, but less than half of these programs remain currently active [4, 8, 9]. There is considerable variability among them in cell sources and preparations, quality control measures, and repopulation methods. In addition, most hepatocyte transplant recipients still receive immunosuppressive regimens modified from solid organ transplant, with lower drug doses and trough goals. Concerns have also been raised about the long-term effects of pretransplant conditioning regimens in children. Clearly, more long-term studies in larger populations are necessary to address these concerns.

3 Technical Considerations in Children

Detailed methods on hepatocyte transplant protocols are presented throughout this book. This section provides a general overview of techniques used in pediatric patients.

3.1 Cell Preparations

Hepatocyte transplantation has the potential to expand the donor pool for children at multiple levels; however in practice, the availability and quality of cells used can be suboptimal. Donor hepatocytes for clinical use are primarily isolated from marginal tissues that were rejected for orthotopic liver transplant. Sources of these non-transplantable organs commonly include steatotic livers, unused segments (from split and reduced size grafts), as well as elderly and non-heart-beating donors; however, more recent application of extended criteria for whole liver donors has further reduced the availability of organs for cell isolation [10, 11]. Unanswered questions remain about cellular senescence and long-term regenerative capacity of hepatocytes isolated from elderly donors and transplanted into pediatric recipients. Additional variables include cold and warm ischemia time. Although cell transplantation can be advantageous when a single donor provides cells to multiple recipients, hepatocytes isolated from marginal livers tend to have reduced cell yield, viability, and function, thereby necessitating a search for multiple donor livers to obtain high quality cells.

Establishing alternative sources of good quality donor hepatocytes is therefore essential. In the spirit of “domino” liver transplantation, hepatocytes isolated from explanted livers may provide another potential source. For example, hepatocytes isolated from explants of patients with metabolic diseases but otherwise normal liver parenchyma could be used for domino cell transplantation, with the native liver compensating for the missing enzyme defect [12]. Explanted diseased livers from low MELD score patients may also have therapeutic potential [13, 14]. Other alternatives to mature human hepatocytes include cells isolated from fetal and neonatal livers [15 – 18]. As differentiation protocols continue to improve, multiple stem cell-derived sources of human hepatocytes (mesenchymal stem cells, biliary cells, amniotic cells) have also been described [19 – 22].

3.2 Patient Preparation

Given the high variability of hepatocyte engraftment into the liver parenchyma, a number of preconditioning regimens have been proposed to provide donor hepatocytes a selective growth advantage over native cells. This is especially helpful in metabolic liver diseases, since there is no massive loss of parenchymal cells to provide regenerative stimulus. Existing preclinical models include partial hepatectomy, portal vein embolization, or the use of drugs or radiation to inhibit native cell growth [23 – 28]. Portal vein embolization is a routine procedure following liver resection for malignancy. Temporary occlusion of the portal vein prior to hepatocyte infusion, with close monitoring of portal pressures, generates an ischemia/reperfusion injury and stimulates a regenerative response. The safety of preparative irradiation in clinical hepatocyte transplantation requires careful assessment in pediatric patients. In theory, hepatic radiation transiently disrupts the sinusoidal endothelial cell barrier, allowing transplanted hepatocytes to engraft and proliferate while arresting the

native cell cycle [29]. Unlike animal models, human liver is highly radiosensitive, and a single 30 Gy dose of whole liver irradiation can cause liver failure due to hepatic no-occlusive disease [7]. Cirrhotic patients are even more susceptible to radiation effects. Concerns of radiation-induced stellate cell activation and fibrosis have also been raised [30]. Although there are no established guidelines for a minimum dose of hepatic radiation in young children, single fractions of 5 Gy delivered to a small portion (<40 %) of the liver to prevent allograft rejection have been reported to be safe in children over the age of 2 years [31]. Clinical trials combining portal vein occlusion and low-dose hepatic irradiation as preconditioning treatment for hepatocyte transplantation are currently in progress.

3.3 Cell Isolation and Transplantation

In general, protocols for isolating human hepatocytes are well established [32]. An aseptic environment, following good manufacturing practice (GMP), is required for large-scale cell isolations. Donor liver tissue is perfused at 37 °C and digested with collagenase, and the cells are purified by centrifugation. Cell yield, viability, and plating efficiency are evaluated. For clinical transplantation, hepatocytes must have a viability >60 % with absence of microbial contamination [7, 8]. Freshly isolated hepatocytes are transplanted immediately after isolation or can be cryopreserved for later use. Cells are matched for ABO compatibility and slowly infused intra-portal to avoid portal hypertension. Routes of administration to the portal system vary. Umbilical vein catheterization is advantageous in neonates and young children. Estimated total cell dose of up to 2×10^8 cells/kg of body weight can be safely given via serial infusions over a period of days, weeks, or months [8]. Cirrhotic patients can be challenging for both infusions and engraftment. Since these patients frequently present with portal hypertension and splenomegaly, the spleen can be a useful ectopic site when cells are transplanted via the splenic artery rather than the splenic vein. This approach is safer than puncturing the splenic pulp, and unlike animal studies, the transplanted cells would not be expected to traverse to the cirrhotic liver.

3.4 Engraftment and Monitoring

Ultimately, successful clinical outcomes for hepatocyte transplantation depend on functioning engrafted cells. As with whole organ transplant, immune suppression is necessary and typically consists of steroid bolus with taper combined with maintenance doses of tacrolimus. Given the limited number of donor cells, elevations in liver enzymes are not reliable biomarkers for acute cellular rejection, making it difficult to titrate antirejection therapy. Similarly, liver biopsy of the native liver is susceptible to sampling error and may appear histologically normal. Of note, it was recently reported that emergence of de novo human leukocyte antigen donor-specific antibodies (DSAs) was associated with graft loss following human hepatocyte transplantation [33]. Still, there remains an unmet need for new biomarkers that better detect rejection of transplanted cells. One alternative approach involves encapsulating hepatocytes with alginate microbeads, which immuno-isolate the donor cells and may even prevent the need for immune suppression [6, 34]. Such encapsulated cryopreserved cells would be ideal for immediate use in children with acute liver failure and could even be administered intraperitoneally if severe coagulopathy prevented access to the portal system. Clinical trials are underway to evaluate this technique in children with acute liver failure [6].

Multiple parameters have also been proposed to monitor sustained engraftment of transplanted hepatocytes. In patients with metabolic liver diseases, following serum metabolites as surrogate noninvasive biochemical markers can parallel correction of the enzyme defect; however, this may not directly correlate with the number of functional engrafted donor cells. If sampled correctly, liver biopsies can be analyzed via analysis of short tandem repeats (STRs) or Y-chromosome FISH (if sex mismatch exists). The risk of morbidity associated with this invasive procedure and the uncertainty of random sampling for a single time point need to be carefully considered in children. Novel techniques for tracking donor cells in animal models include labeling with radioisotopes or magnetic (iron) nanoparticles, which can then be visualized by noninvasive imaging [35 – 38]. Developing reliable noninvasive strategies to follow engraftment of donor hepatocytes over time is essential to demonstrate successful clinical outcomes and the need for future interventions in children.

4 Future Implications and Conclusions

Human hepatocyte transplantation offers children several hypothetical advantages over solid organ transplant. Although considerable progress has been made in the field, more data on efficacy and long-term clinical outcomes in children is needed. As the majority of published studies of clinical hepatocyte transplantation involve pediatric case reports and small series with significant differences in methodology, it will be interesting to learn the results of ongoing clinical trials. The lack of standard protocols for transplanting, monitoring, and preventing rejection of donor cells is still a concern. At best, the ideal pediatric population that currently benefits from hepatocyte transplantation are those with non-life-threatening liver-based metabolic diseases or with acute liver failure; however, in both scenarios, short-term correction as a “bridge” therapy is the most common outcome. Clearly, there are many unanswered questions that must be addressed with both animal models and human studies before widespread clinical use of liver cell therapy in children.

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Table 1
Summary of clinical hepatocyte transplantation in pediatric acute liver failure

Etiology	Age	Effect/outcome	Reference
Drug induced	16 years	Ammonia reduction/death 2 days post-HT	Soriano et al. [40]
	12 years	Ammonia reduction/death 7 days post-HT	
	10 years	Ammonia reduction/death 7days post-HT	
	13 years	Death 4 days post-HT	Strom et al. [41]
	14 years	Ammonia reduction and improved encephalopathy/OLT 1 day post-HT	Fisher and Strom [3]
Idiopathic	3 years5 years	Ammonia reduction and improved encephalopathy in both:Full recovery and immunosuppression weaned Successful bridge to OLT 4 days post-HT	Soriano et al. [40]
	3.5 months	No clear benefit/OLT 1 day post-HT	Sterling et al. [42]
	8 years	Intraperitoneal injection of fetal hepatocytes/full recovery	Habibullah et al. [16]
Viral induced	4 years	Ammonia reduction and improved encephalopathy/intracranial hypertension in day 2	Fisher and Strom [3]
	3 weeks	Death 11 days post-HT	Meyburg et al. [43]

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OLT orthotopic liver transplantation, *HT* hepatocyte transplant

Table 2
Summary of clinical hepatocyte transplantation in pediatric metabolic liver diseases

Disease	Age	Effect/outcome	Reference
Crigler–Najjar syndrome type 1	10 years	50 % reduction in bilirubin/OLT 4 years post-HT	Fox et al. [4]
	8 years	40 % reduction in bilirubin/OLT 20 months post-HT	Darwish et al. [44]
	9 years	30 % reduction in bilirubin/OLT 5 months post-HT	Ambrosino et al. [45]
	1.5 years	40 % reduction in bilirubin/OLT 8 months	Dhawan et al.
	3.5 years	post-HT No clear benefit	[46]
	3.5 years	Lowered serum bilirubin/outcome unknown	Hughes et al. [10]
	8 years	30 % reduction in bilirubin/OLT 11 months post-HT	Allen et al. [47]
	9 years	35 % reduction in bilirubin/OLT waiting list	Lysy et al. [48]
	1 year	25 % reduction in bilirubin/OLT 4 months post-HT	
	2 years	50 % reduction in bilirubin/outcome unknown	Khan et al. [49]
	11 years	20 % reduction in bilirubin/OLT waiting list	Meyburg et al. [43]
	7 months	50 % reduction in bilirubin, psychomotor improvement/ bilirubin stable at 1 year follow-up	Rbes-Koninckx et al. [50]
Alpha-1 antitrypsin deficiency	18 weeks	OLT 2 days post-HT/cirrhosis on explant	Strom et al. [41]
Familial hypercholesterolemia	12 years	Ex vivo gene therapy with autologous cells: No benefit	Grossman et al. [51]
	7 years	6 % reduction in cholesterol and LDL	
	11 years	19 % reduction in cholesterol and LDL	
Factor VII deficiency	3 months	70 % reduction in rFVII requirement/OLT 7 months post-HT	Dhawan et al. [52]
	35 months	70 % reduction in rFVII requirement/OLT 8 months post-HT	
	4 months	Reduction in rFVII requirement/outcome unknown	Hughes et al. [10]
Progressive familial intrahepatic cholestasis 2	32 months	No benefit (cirrhosis established):OLT 5 months post-HT	Dhawan et al. [46]
	16 months	OLT 14 months post-HT	
Phenylketonuria	6 years old	Reduction in Phe levels and improved dietary tolerance up to 3 months post-HT (cells from “domino” GSD1b liver)	Stéphenne et al. [53]
Tyrosinemia type 1	45 days	Improved coagulopathy and bilirubin/OLT 45 days post-HT (cirrhosis on explant)	Rbes-Koninckx et al. [50]
Glycogen storage disease type 1a	6 years	Reduction in hypoglycemic episodes/no hypoglycemic admissions at 1 year follow-up	Rbes-Koninckx et al. [50]
Glycogen storage disease type 1b	18 years	Normal G6Pase activity for 7 months/outcome unknown	Lee et al. [54]
Infantile Refsum's disease	4 years	40 % reduction in pipecolic acid for 18 months/outcome unknown	Sokal et al. [55]
Primary hyperoxaluria type 1	33 months	Reduction in plasma oxalate/liver-kidney transplant 12 months post- HT	Beck et al. [56]

Disease	Age	Effect/outcome	Reference
Urea cycle defects			
Ornithine transcarbamylase deficiency	5 years	Ammonia reduction and protein tolerance/septic death at 42 days post-HT	Strom et al. [57]
	10 h	Ammonia reduction and protein tolerance/OLT 6 months post-HT	Horslen et al. [5]
	14 months	Effect unknown (malpositioned catheter)/OLT 73 days post-HT	Darwish et al. [44]
	14 months	Ammonia reduction, increased urea, and psychomotor improvement/OLT 6 months post-HT	Stéphenne et al. [58]
	1 day	Ammonia reduction, increased urea, and protein tolerance/auxiliary partial OLT 7 months post-HT	Puppi et al. [59]
	6 h	Ammonia reduction, increased urea, normal urine orotic acid/death 4 months post-HT	Meyburg et al. [60]
	9 days	Ammonia reduction, protein tolerance, normal urine orotic acid/OLT waitlist 6 months post-HT	
	5 years	Ammonia reduction, normal glutamine/death 45 days post-HT	Bohnen et al. [35]
	1 day	Ammonia reduction, increased urea, and protein tolerance/auxiliary partial OLT 7 months post-HT and neurologically normal	Mitry et al. [61]
	12 years	Ammonia reduction, increased urea, normal glutamine/septic death 30 days post-HT	Rbes-Koninckx et al. [50]
	11 days	Ammonia reduction/neurologically normal 3 months post-HT	Enosawa et al. [19]
Argininosuccinate lyase deficiency	42 months	Ammonia reduction and psychomotor improvement/OLT 18 months post-HT	Stéphenne et al. [62]
	3 years	Ammonia reduction/outcome unknown	Darwish et al. [44]
Carbamoyl phosphate synthase I deficiency	2.5 months	Ammonia reduction and increased urea /OLT waiting list 11 months post-HT	Meyburg et al. [63]
Citrullinemia	25 months	Ammonia reduced and decreased urea/outcome unknown	Lee et al. (unpublished)
	3 years	Ammonia reduction, increased urea, and protein tolerance/outcome unknown	Meyburg et al. [63]

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OLT orthotopic liver transplantation, *HT* hepatocyte transplant