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Therapeutic Hepatocyte Transplant for Inherited Metabolic Disorders: Functional Considerations, Recent Outcomes and Future Prospects

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Abstract

The applications, outcomes and future strategies of hepatocyte transplantation (HTx) as a corrective intervention for inherited metabolic disease (IMD) are described. An overview of HTx in IMDs, as well as preclinical evaluations in rodent and other mammalian models, is summarized. Current treatments for IMDs are highlighted, along with short- and long-term outcomes and the potential for HTx to supplement or supplant these treatments. Finally, the advantages and disadvantages of HTx are presented, highlighted by long-term challenges with interorgan engraftment and expansion of transplanted cells, in addition to the future prospects of stem cell transplants. At present, the utility of HTx is represented by the potential to bridge patients with life-threatening liver disease to organ transplantation, especially as an adjuvant intervention where severe organ shortages continue to pose challenges.

Keywords

hepatocyte; transplantation; inherited metabolic disease; inborn errors of metabolism; engraftment; expansion; selection

Introduction

Inherited metabolic diseases (IMDs; also referred to as inborn errors of metabolism) represent often rare conditions characterized by accumulation of metabolic intermediates in organs and physiological fluids. Characterization of metabolite accumulation may facilitate identification of the defective pathway(s), many of which are primarily manifest in liver (i.e., α 1-antitrypsin (AAT), tyrosinemia type I, maple syrup urine disease (MSUD), phenylketonuria (PKU), galactosemia, progressive familial intrahepatic cholestasis (PFIC), neonatal hemochromatosis, Wilson's disease, among others). Conversely, other IMDs will manifest additional extrahepatic features (i.e., familial amyloidotic polyneuropathy, primary hyperoxaluria type I (PH), Crigler Najjar syndrome type I (CNSI), familial hypercholesterolemia, urea cycle disorders). While orthotopic liver transplant (OLT) holds significant corrective value for many of these disorders, the outcomes of liver transplantation in IMDs with extrahepatic defects may not be as promising (i.e., erythropoietic protoporphyria, hereditary hemochromatosis, Gaucher disease, nonalcoholic steatohepatitis) (Weiss et al. 2007); (Zhang et al. 2007) (Li 2007)). Trials with

transplantation into recipient liver (i.e., HTx) of the liver's primary functional cell type (hepatocytes) have demonstrated corrective value in clinical and preclinical models of acute liver failure, end-stage liver disease, and in IMDs (Fig. 1).

Liver-related morbidity is the twelfth most common cause of death in the United States, and in 2011 over 16,000 people awaited organ donation (Kochanek et al. 2011), highlighting the expanding need for liver-directed therapeutics. Both cell-culturing techniques and cryopreservation methodologies are showing constant improvements, thereby broadening the potential utility of HTx. Nonetheless, limitations remain in the routine clinical application of HTx: 1) the maintenance of cellular viability post-transplant; 2) immune response/rejection and graft vs. host disease; and 3) engraftment/expansion of transplanted cells into the host liver. Nevertheless, HTx could provide corrective potential for a large spectrum of IMDs, and this potential becomes increasingly relevant in the face of growing donor organ shortages. Clinical studies in disorders such as glycogen storage disorders, and urea cycle defects have already highlighted the corrective capacity of HTx to improve clinical outcomes, and it is reasonable to assume that additional advances with HTx can be expected (Dhawan et al. 2004); (Ribes-Koninckx et al. 2012) (Horslen et al. 2003); (Wan et al. 2013); (Chistiakov 2012); (Yu et al. 2012).

HTX FOR INHERITED METABOLIC DISEASES

Tyrosinemia Type I (Fumarylacetoacetic acid hydrolase (FAH) deficiency)

Mice with genetically-engineered deletion of the *fah* gene represent perhaps the first instance of a genetic disorder in which the feasibility of HTx was documented (Karnezis et al. 2001); (Erker et al. 2010); (Paulk et al. 2012). Grompe and colleagues successfully exploited *fah* deficiency in the distal portion of the tyrosine metabolic pathway to provide an engraftment advantage for exogenously administered *fah*^{+/+} cells to *fah*-deficient mice. In human tyrosinemia type I, patients invariably progress to early morbidity from hepatocellular carcinoma, a finding recapitulated in the murine model. Intervention with nitisinone, an inhibitor of the upstream formation of homogentisic acid in the tyrosine pathway, prevents the formation of hepatotoxic fumarylacetoacetic acid in patients and mice, a life-saving intervention in both. To achieve successful HTx in this model, *fah*-deficient mice were reared with concomitant NTBC treatment, and *fah*^{+/+} hepatocytes applied via intrasplenic administration with staged removal of nitisinone intervention. With this approach, the liver of *fah*-deficient mice could be ~90% repopulated with *fah*^{+/+} cells via a single exogenous cell administration (Hamman et al. 2005); (Al-Dhalimy et al. 2002). In an FAH-deficient newborn, HTx successfully bridged the patient to OLT (Ribes-Koninckx et al. 2012).

Phenylketonuria (Phenylalanine hydroxylase (PAH) deficiency)

Phenylketonuria (PKU) results from mutations in the enzyme phenylalanine hydroxylase (PAH) located on the long arm of chromosome 12. High levels of phenylalanine have been suggested to saturate the large neutral amino acid (LNAA) transporter, LAT1, at the blood brain barrier. The monoamine neurotransmitters dopamine and serotonin may be depleted due to insufficient levels of their essential precursors, tyrosine and tryptophan, which are also LNAAs employing the LAT1 for brain access. Dopamine and serotonin regulate important biological processes including movement, mood, speech, and appetite, as well as many other processes. Moreover, depletion of tyrosine can impact noradrenergic neurotransmitters, including epinephrine and norepinephrine. Additionally, oligodendrocytes responsible for assembly and maintenance of the myelin sheath surrounding neurons are sensitive to high brain phenylalanine, and the hypomyelination, demyelination, lesions,

white matter abnormalities, and cognitive impairment observed in PKU have been attributed to oligodendrocyte damage associated with hyperphenylalaninemia (Dyer 1999).

Current Treatment for PKU

Standard intervention requires strict adherence to a protein restricted, low phe diet supplemented with low phenylalanine formula for protein supplementation (MacDonald 2000). Phe is an essential amino acid, and even diet-adherent patients may experience metabolic flux and suboptimal outcomes such as cognitive deficits. A number of new therapies are currently in phase I–III clinical trials in patients (www.clinicaltrials.gov), including sapropterin (Kuvan™; Phenoptin™), phenylalanine ammonia lyase (PAL) enzyme therapy, and dietary administration of glycomacropeptide, a whey by-product devoid of phenylalanine (LaClair et al. 2009); (Bélanger-Quintana et al. 2011). Sapropterin essentially represents cofactor therapy, and is effective only in a portion of PKU patients undergoing intervention (Harding 2010).

Clinical and Preclinical HTx in PKU

Hamman and colleagues (2005) demonstrated that 5–10% repopulation of double mutant *fah*^{-/-}/*Pah*^{enu2} mice was incapable of completely correcting phenylalanine levels. As described above, the *fah* background was employed to provide an engraftment advantage (Orejuela et al. 2008). Transplant of double mutant subjects with *fah*^{+/+}/*pah*^{+/+} hepatocytes was predicted to substantially enhance engraftment (Al-Dhalimy et al. 2002); (Overturf et al. 1999). Partially corrected mice revealed significant reduction of serum PHE levels (<700 µM) when hepatocyte engraftment exceeding 5%. Repopulation levels of 20% corrected PHE in a single subject (85–175 µM). The authors also employed heterozygous (*Pah*^{enu2/+}) donors and found similar levels of engraftment correlated with partial serum PHE correction (Hamman et al. 2005). In a follow-up study (2011), the same group transplanted the double mutant model with 1–4×10⁵ *fah*^{+/+}/*pah*^{+/+} hepatocytes administered intrasplenically. These investigators found that 3–10% engraftment partially corrected blood PHE levels in deficient mice, while >10% transplant engraftment resulted in normalization. Hamman and colleagues concluded that the absolute PAH activity/cell was a less critical variable than the actual number of PAH expressing cells (Hamman et al. 2011).

Only limited clinical studies of HTx have been piloted in PKU patients. In a study by Stéphenne et al (2012), a 6-year-old boy diagnosed with tetrahydrobiopterin non-responsive PKU was referred for HTx due to chronic dietary non-compliance. Blood phe was 564 ± 264 µmol/L (exceeding the prescribed upper limit of 360 µmol/L). He received domino transplant with hepatocytes isolated from the explant of a patient with glycogen storage disease type 1 (GSD1a; see below), but this failed to achieve clinical improvement. A second cellular infusion with hepatocytes derived from a “non-disease” liver was more successful, decreasing the half-life of Phe from 43 to 19 hours and leading to reduction in Phe levels for a duration of 3 months (Stéphenne et al. 2012). Of interest, an active trial of HTx in phenylketonuria is open and currently recruiting (www.clinicaltrials.gov), sponsored by the University of Pittsburgh and Children’s Hospital of Pittsburgh of UPMC.

Maple Syrup Urine Disease (MSUD)

The branched-chain amino acids leucine, isoleucine, and valine undergo oxidative metabolism beginning with two steps common to all three amino acids: 1) transamination via branched-chain amino acid aminotransferase; and 2) subsequent oxidative decarboxylation catalyzed by branched-chain α-ketoacid dehydrogenase complex (BCKDH). Patients with MSUD harbor autosomal-recessively inherited mutations in any of three subunits involved in the oligomeric BCKDH complex: a) decarboxylase (E1 subunit); b) transacetylase (E2 subunit); or c) lipoamide dehydrogenase (E3 subunit). Defects in the

E3 subunit also lead to inherited deficiencies in pyruvate and α -ketoglutarate dehydrogenase deficiencies, since E3 is a subunit common to these enzyme complexes as well. BCKDH deficiency results in accumulation of both BCAA and branched-chain keto acids in blood and tissues of MSUD patients. Increased concentrations of BCAA/BCKA, especially leucine and its transamination product α -ketoisocaproic acid (KIC), are particularly toxic in brain (Zinnanti et al. 2009); (Strauss et al. 2010), and may associate with neurotransmitter deficiency, neurological dysfunction and other pathomechanisms (Schönberger et al. 2004). As is the case for PKU, the BCAAs are also LNAAs that can lead to saturation of the LAT-1 system when accumulated to high levels in the peripheral blood (Zinnanti et al. 2009). Accumulation of KIC enhances transamination of alanine (leading to pyruvate and lactate accumulation) and glutamate (increasing generation of α -ketoglutarate). Glutamate is the direct precursor of inhibitory GABA, which may also be depleted (Zinnanti et al. 2009). As for PKU, MSUD treatment is composed of dietary protein restriction and formula supplementation to ensure adequate intake of BCAAs necessary for somatic growth and brain development. Orthotopic liver transplantation (OLT) is particularly beneficial in classical MSUD where residual BCKDH activity is very low and outcomes quite poor (Strauss et al. 2006a); (Mazariegos et al. 2012).

Preclinical Characterization of HTx in *msud* Mice

Homanics and Skvorak developed murine models of MSUD (2006). Complete ablation of the E1 α subunit of BCKDH led to a model of classical MSUD with extremely truncated survival (Zinnanti and Lazovic 2012). This model was attenuated through transgenic knock-in of the human E1 α subunit, resulting in a model termed intermediate MSUD (*imsud*), with ~ 5–7% of residual liver BCKDH and an extended lifespan (Homanics et al. 2006). The observation that OLT was essentially corrective in classical MSUD led Skvorak and colleagues to preclinically examine HTx in *imsud* mice. These investigators injected BCKDH-replete hepatocytes directly into the hepatic mass of neonatal *imsud* pups, employing the rapid expansion characteristics of neonatal liver as a potential mechanism to favor engraftment. Enhanced expression of BCKDH activity in liver of transplanted *imsud* subjects was associated with improved biochemical parameters and extended lifespan. Notably, significant corrections were observed in brain neurotransmitter abnormalities as well (Skvorak et al. 2009a); (Skvorak et al. 2009b). The same authors subsequently extended these studies employing human amniotic epithelial cells in *imsud* subjects, taking advantage of the stem cell-like characteristics of these cells (Skvorak et al. 2013a; Skvorak et al. 2013b).

Urea Cycle Disorders

The urea cycle disorders encompass carbamoyl phosphate synthetase I (a mitochondrial regulatory enzyme), ornithine transcarbamoylase (also mitochondrial), argininosuccinate synthetase and lyase, and arginase (cytosolic localization). Deficiency of any of these enzymes blocks urea production, resulting in hyperammonemia and accumulation of other nitrogenous intermediates. Much of the pathology related to these disorders is associated with hyperammonemia, including lethargy, tremors, athetosis, convulsions, spastic (para)plegia, and ataxia. Biochemical and anthropomorphic findings in these patients include protein intolerance/avoidance, growth retardation, respiratory alkalosis and vomiting, and particularly in infants an inability to regulate body temperature and/or breathing. While dietary and pharmacological treatment of urea cycle disorders significantly improves prognosis (estimated incidence 1:8,000) (Meyburg et al. 2009), severe cases of urea cycle disorder require liver transplantation at a young age to improve prognosis (Campeau et al. 2010). Accordingly, HTx has been attempted in the majority of the urea cycle disorders (Meyburg and Hoffmann 2010).

HTx in Urea Cycle Disorders

Since the urea cycle is almost entirely localized to hepatic tissue, defects in this cycle are excellent candidates for HTx as well as OLT. The first attempted transplant of hepatocytes into a patient with a urea cycle disorder was performed by Strom and colleagues (1997) in a patient with OTC, although the patient died by day 43 of life in hyperammonemic coma. In 2003, this was followed by a further demonstration of HTx in an newborn male with severe OTC deficiency. HTx was begun on DOL 1, and by DOL 23 he had received 11 infusions totaling $\sim 5 \times 10^9$ hepatocytes derived from 7 different donors. Protein intake was gradually increased (1.5 g/kg from formula, 0.5 g/kg from breastmilk) until DOL 31 when onset of hyperammonemia was noted and dietary protein intake was subsequently reduced (1 g/kg). Metabolic decompensation was attributed to inadequate immunosuppression, and further cell infusions beyond this time point were acutely rejected (Horslen et al. 2003).

The efficacy of cryopreserved hepatocytes as a source of administered cells was first documented in OTC patients (Mitry et al. 2004)(Stéphenne et al. 2005). Three hepatocyte isolations (80% of segment IV, resected proximal to the middle hepatic vein, or segment IV with the caudate lobe) were cryopreserved and infused into three male OTC patients (age 18 to 33 years). The total hepatocyte infusion number was $\sim 10^8$ per patient with 80 – 95% initial viability. Ammonia levels and serum urea improved, and provided proof-of-principle for the utility of segment IV as a viable source of hepatocytes (Mitry et al. 2004). A 14-month-old OTC patient received 10 hepatocyte infusions over 1.5 months with the goal of bridging to OLT (OLT performed 6 months after the first infusion). In total, the patient received 10^9 cryopreserved hepatocytes. Ammonia levels were modestly improved or static. Two hyperammonemic episodes occurred during the infusion protocol, and liver enzymes (aspartate and alanine aminotransferase) increased after one infusion; nonetheless, blood urea levels and psychomotor development were improved (Stéphenne et al. 2005).

Additional HTx studies reported successful outcomes in OTC deficiency that coupled HTx as a bridge to auxiliary partial orthotopic liver transplantation (APOLT) (Puppi et al. 2008) (Legido-Quigley et al. 2009). In the latter, partial correction of plasma and urine metabolic disturbances was rapidly monitored employing NMR spectroscopy. Despite promising outcomes in this study, recurrent bouts of sepsis in the proband thwarted long-term correction and APOLT was required to achieve long-lasting metabolic control. In other urea cycle disorders, sequential HTx in a child with argininosuccinate lyase (ASL) deficiency led to long-term metabolic control, psychomotor catch-up, and 3% of control ASL activity in biopsied liver obtained 8 months post HTx (Stéphenne et al. 2006). Meyburg and coworkers (2009) employed a single neonatal donor liver for cell isolation and HTx in four patients with urea cycle disorders, including CPS, OTC and ASS (argininosuccinate synthetase; type I citrullinemia) deficiencies. All patients showed metabolic improvements and were effectively bridged to OLT, although the patient with prenatally diagnosed OTC died within 6 months from severe metabolic crisis (Meyburg et al. 2009). It is worth noting that Cytonet GmbH & Co. (www.cytonetllc.com) is currently pursuing an FDA-approved clinical trial of HTx in urea cycle disorders, although preliminary outcomes have not been presented at this time.

Crigler-Najjar (CN) Syndrome

The Crigler-Najjar syndrome, first described in 1952, represents a heritable form of jaundice resulting from high levels of unconjugated bilirubin and, untreated, leading to severe neurotoxicity in the central nervous system (Crigler and Najjar 1952). Bilirubin represents a by-product of heme metabolism generated during the normal recycling of red blood cells, and it is cleared in the endoplasmic reticulum of hepatocytes by bilirubin-UDP-glucuronosyl transferase (UGT1A1). Bilirubin accumulates in the normal turnover of hemoglobin, and

increases in serum when production from heme exceeds 4 mg/kg/day. Two forms of the disease exist, type I and II, differing in age of onset (birth for type I, later in life for type II; also referred to as Arias syndrome) and autosomal-recessively inherited mutations in the UGT gene are known for both subtypes (Sampietro and Iolascon 1999).

Types I and II CN syndrome are characterized clinically by hepatic and CNS pathology including jaundice, hypotonia, oculomotor palsy, kernicterus and encephalopathy. Daily treatment of the type II disease with phenobarbital effectively lowers serum bilirubin via induction of UGT expression. Additionally, blood transfusion can lower plasma bilirubin, and calcium chelators can complex and lower bilirubin in the gut. CN type I, however, lacks appreciable UGT1A1 activity associating with very high levels of unconjugated bilirubin. CN I patients rely upon phototherapy, which is dependent upon exposure to high intensity blue light on a regular basis, depending upon patient age and the nature of the gene mutation (Strauss et al. 2006b); (Sampietro and Iolascon 1999). The only effective cure for CN I is liver transplantation, including APOLT (Rela et al. 1999) and living donor liver transplantation (Ozçay et al. 2009); (Ribes-Koninckx et al. 2012).

The Gunn Rat

Gunn first reported on a Wistar rat strain that exhibited jaundice and a recessive inheritance pattern. Subsequently, deficiency of UGT1A1 was identified, representing a relevant phenocopy of CN I syndrome (McCandless 2011). A number of preclinical HTx studies have shown efficacy in this model, including application of immortalized hepatocytes, bone marrow-derived cells, as well as intrasplenic administration of both fetal and adult hepatocytes (Kawashita et al. 2008); (Muraca et al. 2007); (Cubero et al. 2007); (Guha et al. 2002). In these studies, engraftment advantage was achieved by irradiation, partial hepatectomy, or induction of ischemic/reperfusion injury to damage endogenous tissue. More recently, single liver lobe HTx in this model, employing preparative irradiation and concomitant application of HGF, have shown correction of bilirubin excretion and associated jaundice (Zhou et al. 2012). Studies in the Gunn rat have provided some of the most important proof-of-principle data to support human HTx trials. In this model, it has been estimated that repeated hepatocyte infusion could result in 5–10% of hepatic mass replacement, without the requirement for irradiation or partial hepatectomy (Horslen and Fox 2004).

Clinical Application of HTx in CN I

In a 10 year-old female with confirmed CN I disease, 7.5×10^9 hepatocytes were administered via portal vein access over 15 hours. Immunosuppression consisted of corticosteroids, prednisone, and tacrolimus. Partial correction was observed 11 months post-transplant that required 6–7 hours of phototherapy/day (reduced from 10–12 hr/day) and an improved total serum bilirubin level (post-transplant, 14 mg/dL; pre-transplant, ~ 26) (Fox et al. 1998). A 9-year-old male with confirmed CN type I boy received 7.5×10^9 hepatocytes from a split-liver cadaveric donor, without infusion complications. Two weeks post-transplant he was supplemented with phenobarbitone, and the serum bilirubin was decreased by 30% in comparison to pre-transplant level. Parental noncompliance for prescribed phototherapy coupled with inadequate immunosuppression ultimately led to OLT (Ambrosino et al. 2005). Lysy et al (2008) reported HTx in two pediatric CN I patients. Patient 1 received 6.1×10^9 hepatocytes at age 9 years using a series of 18 infusions (delivered via catheter to the jejunal vein) over a 5 month timeframe. Serum bilirubin levels decreased approximately 30% in comparison to pre-transplant levels, but spiked 6 months post-transplant and he progressed clinically to OLT. The second patient (a female) received 14 infusions of 2.6×10^9 hepatocytes at 1 year of age (Lysy et al. 2008). The total hepatic mass transplanted was ~9%; serum bilirubin fell approximately 25% and skin jaundice

rapidly improved. Unfortunately, viral infection led to rapid deterioration which required OLT.

Familial Hypercholesterolemia

An autosomal dominant disorder, familial hypercholesterolemia (FH) results from mutations in either the low density lipoprotein receptor gene (LDLR) or apolipoprotein B-100 gene (APOB). The frequency of FH is ~1/500 in caucasians, but higher in certain populations due to founder effects. Homozygous mutations are particularly deleterious, leading to blood cholesterol levels that exceed 1000 mg/dL (Gautschi et al. 2012). FH is characterized by significantly increased plasma total cholesterol and low density lipoprotein cholesterol, xanthomas (cholesterol-rich deposition in tendons and joints) and cardiovascular disease. Interventional targets include reduction of saturated fat intake and lowering of blood cholesterol. Cholesterol-lowering drugs (statins) and cholesterol-sequestering agents remain the mainstay of therapeutic intervention. Extracorporeal apheresis and hemodynamic filtration may be required in instances of hypercholesterolemia that is non-responsive to medications (Gautschi et al. 2012).

Preclinical HTx in the Watanabe Hyperlipidemic (WHHL) Rabbit

The WHHL rabbit was developed by Yoshio Watanabe (Watanabe 1980), and these animals demonstrate hypercholesterolemia due to a deficiency of LDL receptors, with lipoprotein metabolism quite comparable to humans. WHHL rabbits have undergone HTx with *ex-vivo* transfected hepatocytes. LDLR deficiency was corrected in harvested hepatocytes employing recombinant retrovirus administration. Subsequently, harvested cells were administered to WHHL rabbits via portal vein infusion at $1-2 \times 10^8$ cells per subject (up to 4% of hepatic mass). Total serum cholesterol declined significantly within 5 days post-transplant (70% reduction compared to pre-treatment level). Lipid abnormalities (LDL, VLDL) resolved within one week post-transplant and remained within normal levels for 6 months (Kakaei et al. 2009); (Okura et al. 2011)(Mohamadnejad et al. 2010)(Allian-Sauer and Falko 2012).

Clinical HTx in FH

HTx in FH was first achieved using *ex vivo* autologous hepatocytes transfected with recombinant retroviral LDLR (Grossman et al. 1994). To improve transplant-cell engraftment and proliferation, and to obtain hepatocytes for transformation, the recipient underwent left lateral segment lobectomy prior to cell administration. The recipient (homozygous FH) demonstrated modest improvements in LDL/HDL (30% improvement) levels 18 months post-surgery (Grossman et al. 1994); (Burlina 2004).

Refsum Disease, a Disorder of Peroxisome Biogenesis

Refsum disease results from defects in the α -oxidation of long chain fatty acids or in catabolic enzymes within the peroxisome. Inherited peroxisomal disorders group into either single metabolic enzyme disorders or peroxisome biogenesis disorders (PBD) (Kim 2009), with a cumulative incidence of ~1:20,000 live births. Prior to transport to the mitochondria for subsequent β -oxidation, pristanic acid is generated from phytanic acid via α -oxidation. The enzyme deficiency normally associated with Refsum's disease, phytanoyl-CoA 2-hydroxylase, catalyzes the conversion of phytanoyl-CoA to 2-hydroxyphytanoyl-CoA. Mutations in hydroxyphytanoyl-CoA lyase are also associated with Refsum's disease. Clinical and biochemical manifestations of Refsum disease include marked elevation of plasma phytanic acid, retinitis pigmentosa, blindness, anosmia, deafness, sensory neuropathy, and ataxia. Treatment consists of elimination of phytanic acid from the diet, plasmapheresis (in conjunction with albumin replacement), and/or cascade filtration to

reduce blood phytanic acid levels. Additional treatment options include administration of dermatological drugs that soften the skin and ultimately upregulate α -hydroxylation of phytanic acid to a limited extent. OLT decreases phytanic acid and very long chain fatty acids in serum and improves elimination of abnormal plasma bile acids (Van Maldergem et al. 2005)(Zolotov et al. 2012).

HTx in Refsum Disease

A 4 year-old female diagnosed with Refsum disease manifested advanced neurological morbidity, which obviated the potential therapeutic benefit of OLT. She was subsequently scheduled for HTx. Hepatocytes were derived from the unused left liver lobes of two compatible male donors. The patient received two transfusions of 10^9 fresh hepatocytes initially, followed by two additional administrations of 10^6 cryopreserved hepatocytes on days 3–5. Biochemical improvements included a 30% decrease in blood very long chain fatty acids, $\sim 1/3$ decrease in plasma pipelicolic acid (an additional biomarker of peroxisome biogenesis deficiency), and improvements in blood dihydroxycoprostanic acid, the precursor to chenodeoxycholic acid. Visual and auditory evoked potentials showed no improvement, although somatic growth improved as did locomotor activity (Sokal et al. 2003).

Wilson's Disease

Wilson's disease results from mutations in the ATP7B-encoding copper transporting ATPase (P-type) 2, resulting in copper accumulation in hepatocytes and the central nervous system. Neuropsychiatric morbidity may develop in the 2nd and 3rd decades of life. Pathology may manifest as clinical liver disease, but timely intervention can retard disease progression and prevent selected pathological features, including corneal Kayser-Fleischer rings (copper deposition in the Descemet membrane of the cornea), elevated urinary copper excretion, reduced circulating ceruloplasmin, and elevated hepatic copper stores (Fenu et al. 2012). Copper is an essential trace metal required for growth and development, and as cofactor for numerous cuproproteins. Cytotoxicity results from excess copper levels associated with the production of reactive oxygen species (Peña et al. 1999). More than 500 pathogenic mutations have been identified in Wilson's disease, with an estimated incidence of $\sim 1:30\text{--}50,000$ live births (Olivarez et al. 2001). Intervention includes lifelong adherence to a low copper diet (avoidance of high copper foods such as chocolate, dried fruit, liver, nuts, and mushrooms), copper-chelators including penicillamine or trientine, and zinc acetate (which blocks copper absorption in the GI).

HTx in Wilson's Disease

HTx has been extensively investigated in the Long Evans Cinnamon (LEC) rat model which harbors an *atp7b* point mutation and associated copper toxicosis of the liver (Yoshida et al. 1996)(Irani et al. 2001); (Malhi et al. 2002b); (Park et al. 2006); (Joseph et al. 2009). A variety of preconditioning approaches have been employed in these studies, including administration of retrorsine, partial hepatectomy, and the use of intraperitoneal tacrolimus administration to immunosuppress recipient animals. Outcome measures have included serum aminotransferases, liver, bile and copper output, ceruloplasmin oxidase activity, and quantitation of *atp7b* poly A⁺ RNA levels. Long-term analyses of metabolic correction ranged from 70–90% in most of these studies. In the most recent study, liver irradiation pretreatment associated with cholic acid (bile salt) administration prior to HTx resulted in 75% of test animals achieving long-term metabolic improvements (Joseph et al 2009). In corrected animals, fulminant hepatitis, chronic inflammation, and cholangiofibrosis, often observed in untreated LEC animals, were absent and liver histology was improved (Irani et al. 2001). Joseph and colleagues (2009) suggested that bile-salt-induced liver damage was

very effective as a preparative HTx treatment for this model, in view of the pre-existing hepatic damage already present.

Primary Hyperoxaluria (PH)

Autosomal-recessively inherited mutations in the alanine-glyoxylate aminotransferase (AGT) gene, catalyzing the transamination of glyoxylate to glycine, give rise to primary hyperoxaluria (PH). The inherited enzyme defect leads to excessive conversion of glycine to oxalate, the latter depositing (oxalosis) in bones, heart, retina, arteries, nerves, and predominantly in the kidney. Accordingly, end-stage renal failure occurs in ~1/2 of patients by the age of 15 years. Partial liver transplant is insufficient to reduce oxalate, since remaining hepatocytes continue producing oxalate. OLT is metabolically corrective, but where systemic oxalosis has progressed to end-stage renal failure, combined liver-kidney transplantation is mandated (Ellis et al. 2001)(Hoppe 2012)(Beck et al. 2012).

Preclinical and Clinical HTx in PH

Guha and colleagues (2005) developed preparative irradiation and hepatocyte mitotic stimulation procedures in order to evaluate the feasibility of HTx in PH. These approaches were applied in a murine PH model, the *agxt*^{-/-} mouse, and were sufficient to correct urinary oxalate excretion via intrasplenic cell administration. Mitotic stimulation was achieved via application of adenoviral-based HGF (Guha et al. 2005). Beck and colleagues (2012) extended these results by piloting HTx as a bridge to OLT in a 15 month old female patient with severe PH. Intraportal administration of 10⁹ cryopreserved cells was undertaken, over a 6 day period, with tacrolimus as immunosuppressant. Plasma oxalate continuously decreased, with long-term values (8 weeks to 11 months) significantly lower than pre-HTx values. However, due to vascular complications and graft dysfunction the patient underwent OLT at 12 months. During the period of plasma oxalate correction, there was marked clinical improvement that effectively bridged the patient to combined liver-kidney transplantation (Beck et al. 2012); (Jiang et al. 2008)

Progressive Familial Intrahepatic Cholestasis (PFIC)

A heterogeneous class of autosomal-recessively inherited disorders, the subtypes of PFIC associate with different mutations in bile forming hepatocellular transport-systems. PFIC1 and PFIC2 are early onset, while PFIC3 may arise later in childhood or adulthood. PFIC presents with unrelenting hepatocellular cholestasis, pruritis and jaundice (Jacquemin 2012). Extensive fibrotic lesions often associate with end-stage liver disease in PFIC. Molecular defects in ATP8B1 (encoding FIC1 protein) and ABCB11 (encoding the bile salt export pump (BSEP) protein) represent the etiological underpinnings of PFIC 1 and 2. Bile salt secretion is severely impaired in both, as expected. PFIC 3 associates with defects in ABCB4, which encodes multidrug resistance 3 protein (MDR3) and impairs biliary phospholipid secretion. Bile salt intervention (e.g., ursodeoxycholic acid) represents a mainstay of therapy to mitigate liver damage, and biliary diversion may be beneficial to slow disease progression (Jankowska and Socha 2012). Nonetheless, most cases of PFIC progress to OLT.

Preclinical and Clinical HTx in PFIC

Targeted ablation of the murine orthologue of MDR3, *mdr2*, yielded a representative model of PFIC with hepatic lesions indicative of primary sclerosing cholangitis (Popov et al. 2005). Male *mdr2*^{-/-} mice were injected intrasplenically with 10⁶ hepatocytes, and 1 week post-HTx both transplanted and control mice were placed on a diet of 20% casein diet or the same supplemented with 0.3% cholate. Mice were harvested for tissue collection (bile, liver) up to 50 weeks, as well as for assessment of pathology and engraftment efficiency (De Vree

et al. 2000). Engraftment efficiency of exogenous cells (*mdr2*^{+/+}) was enhanced by bile salt supplementation. Unfortunately, cholate supplementation induced development of multiple hepatic tumors within 1 year post-HTx. Conversely, transplanted animals on casein-only diet repopulated more slowly and improvement of liver pathology was significantly curtailed. These studies provided proof-of-principle for HTx in PFIC type 3 (De Vree et al. 2000). Nonetheless, there is still controversy as to whether these animal models are a faithful phenocopy of the human disease.

Bile salt export pump (BSEP) deficiency (PFIC-2) is the result of inherited mutations in the *ABCB11* gene, coding for an ATP-binding cassette transporter. The BSEP protein is critical for bile transport and maintenance of liver function. In the *Bsep*^{-/-} null mouse model, Chen and colleagues (2012) found that feeding of cholic/ursodeoxycholic acids to *Bsep*^{+/+} and *Bsep*^{-/-} subjects resulted in increased proliferative capacity in wild-type subjects, but cholestasis and apoptosis in *Bsep*^{-/-} subjects. The observed differential toxicity was employed as a preparative preconditioning technique for HTx in the same subjects. This approach resulted in biliary total bile acid levels in *Bsep*^{-/-} mice that was 80% of normal, providing valuable preclinical data for future trials of HTx in PFIC-2 patients (Chen et al. 2012). Human HTx has been reported in two PFIC-2 patients, with partial recovery of metabolic functions, but both patients rapidly progressed to OLT (Quaglia et al 2008).

Glycogen Storage Diseases (GSD)

Glycogen storage disease type I (GSDI) represents an autosomal-recessively inherited disorder resulting in the accumulation of hepatic glycogen and fat. The phenotype encompasses hypoglycemia, hepatomegaly, lactic acidosis, hyperuricemia, and hyperlipidemia. Mutations in the *G6PC* (glucose-6-phosphatase) gene at locus 17q21.31 give rise to GSDIa, whereas mutations in the *SLC37A4* at locus 11q23.3 produce GSDIb, with an approximate 4:1 prevalence of GSDIa vs1b. GSDIb patients manifest hypoglycemia, growth retardation, hepatomegaly, nephromegaly, hyperlipidemia, hyperuricemia, and lactic acidemia. Chronic neutropenia and functional deficiency of neutrophils and monocytes in GSD 1b often leads to transplant with granulocyte colony stimulating factor (Ribes-Koninckx et al. 2012). *G6PC* mutations result in loss of glucose-6-phosphatase activity, which curtails glycogenolysis and results in the typical hypoglycemia observed in GSDIa. Conversely, *SLC37A4* mutations lead to impaired glucose-6-phosphate (*G6P*) translocase activity, preventing migration of *G6P* to the endoplasmic reticulum and resulting in hypoglycemia and hepatomegaly. The cumulative frequency of all GSDs is ~ 1 in 20,000–25,000 live births, with GSDI (a or b) occurring in ~1 in 100,000 live births. Treatment is usually dietary or occasionally pharmacologic; nonetheless, in severe cases, liver transplantation has been performed.

HTx in GSD1a

HTx was undertaken in a 47-year-old female with GSD1a who suffered severe fasting hypoglycemia. The clinical history included diagnosis at 3 years of age following presentation with hepatomegaly, hypoglycemia, hypertriglyceridaemia, hyperuricaemia, and lactic acidosis. *G-6-Pase* activity was undetectable in liver biopsy. Frequent meals, including nocturnal nasogastric feeds and uncooked corn starch meals every 3 hrs were employed to maintain blood glucose. Inability to comply with dietary requirements and an inability to maintain blood glucose > 3.5 mmol/L (normal 3.7–6.1) eventually led to HTx intervention. Hepatocytes (10⁹) were infused via an indwelling catheter inserted into the portal vein, which was followed by a cocktail of immunosuppressants including mycophenolate mofetil, tacrolimus, and steroids. Nine months post-HTx immunosuppression was limited to tacrolimus, and she was able to consume a normal diet and fast up to 7h without overt evidence of hypoglycemia (Muraca et al. 2002).

HTx in GSD1b

An 18-year-old male with GSD1b, referred for HTx, displayed severe growth retardation, epistaxis, hepatomegaly, hypoglycemia, and neutropenia. Hepatocytes were obtained from cadaveric whole livers derived from 2 donors, and were infused at days 1, 7, and 30 (each infusion, 10^9 cells). Post-transplant, the patient maintained normal blood glucose and neutrophil levels and discontinued cornstarch meals. After one year, the patient displayed pubertal development (significantly delayed pre-HTx) and a 12 cm gain in height. Nonetheless, hepatosplenomegaly persisted. Two liver biopsies were undertaken post-HTx in order to assess G-6-P translocase activity. Since G-6-P translocase defects in GSD1b impede transport of G-6-P into the endoplasmic reticulum and G6Pase is inactive in the cytoplasm, G-6-Pase activity is increased post-sonication with substrate accessibility (in GSD1, G-6-Pase activity is well below the control presonation range of 5.9–93.0 nmol/min). Translocase values (0, 20 and 250 days post-HTx) showed increases in G-6-Pase ER internalization from 0.18 (baseline) to 21 and 18 nmol/min/mg protein, pre- and post-sonication, respectively. These results provide evidence for engraftment and functional activity (Lee et al. 2007).

Conclusions from HTx in Metabolic Disorders

Results for HTx in a number of metabolic (and non-metabolic liver diseases) have underscored the utility of this procedure to bridge the patient with life-threatening liver disease to orthotopic liver transplantation (Table I). Two critical obstacles remain to be overcome to bring HTx to full clinical utilization: 1) transplanted cells, which are gene-replete, must have an engraftment advantage over endogenous gene-defective cells (if not an advantage, then minimally some type of selective advantage); and 2) transplanted cells must have expansion advantage, and this is particularly of concern with immunorejection of the transplanted cells (Puppi et al. 2012). Distinctions between these terms (engraftment, expansion) may be small, and likely in the clinical setting the goals for both are overlapping. While HTx studies in animal models have shown utility, these promising results have not yet translated to the clinic, for the above reasons. Selected disorders in which the gene defect leads to cellular apoptosis in the absence of intervention (e.g., *fah*^{-/-} mice) confer a distinct potential engraftment advantage to exogenous cells that is not universally available in other metabolic liver diseases. Also, endogenous liver damage, such as that found in the LEC rat model of Wilson's disease provide a unique venue amenable to HTx, due to the nature of the pre-existing damage. Limited recent studies have begun to highlight the potential utility of amniotic epithelial cells from human as a preclinical donor model for metabolic liver diseases (Skvorak et al 2012; (Ordóñez and Goldstein 2012). Many stem cells show strong immunosuppressive qualities and the pluripotent nature of these cells provides the opportunity for differentiation to mature hepatocytes. Although further studies continue to underscore the potential for HTx to treat metabolic liver diseases, until such time as effective and non-invasive protocols are developed that can enhance engraftment and expansion of exogenous cells, HTx will remain in the short term primarily a “bridging” protocol to OLT and APOLT.

Selected Abbreviations

AGT	Alanine glyoxylate transaminase
APOLT	Auxiliary Partial Orthotopic Liver Transplant
ASL	Argininosuccinate lyase
ASS	Argininosuccinate synthetase

BCAA	Branched-chain amino acids
BCKDH	Branched-chain ketoacid dehydrogenase complex
CNSI	Crigler-Najjar syndrome type I
CPSI	Carbamoyl phosphate synthetase I
FAH	Fumarylacetoacetate hydrolase deficiency (tyrosinemia type I)
GSD	Glycogen storage disease
HDL	High density lipoprotein
HGF	Hepatic growth factor
HTx	Hepatocyte transplantation
KIC	α -Ketoisocaproic acid
LDL	Low density lipoprotein
MSUD	Maple syrup urine disease
NAGS	<i>N</i> -acetylglutamate synthetase
OLT	Orthotopic liver transplantation
OTC	Ornithine transcarbamylase
PFIC	Progressive familial intrahepatic cholestasis
PHE	Phenylalanine
PH	Primary Hyperoxaluria
PKU	Phenylketonuria
UGT	bilirubin-UDP-glucuronosyl transferase

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(Modified from Thibodeau GA, Patton KT. *Anatomy & physiology*, ed 5, St Louis, 2003, Mosby.)

Fig.
Gross architecture of a hepatic lobule, showing key structural elements such as hepatic plates, veins and ducts, canaliculi and hepatocytes. Modified from McCance KL, Huether SE, Brashers VL, Rote NS (2010) *Pathophysiology: The Biologic Basis for Disease in Adults and Children* (ISBN 978-0-323-06584-9), with Permission.

Table I

Heritable Metabolic Disorders for which Hepatocyte Transplantation has been Attempted (Preclinically or Clinically)

Disorder	Affected Enzyme	OMIM Entry	Preclinical/Clinical Approach
Tyrosinemia type I	Fumarylacetoacetate hydrolase	276700	Preclinical/Clinical
Phenylketonuria	Phenylalanine Hydroxylase	261600	Preclinical/Clinical
Maple syrup urine disease	Branched Chain Ketoacid Dehydrogenase	248600	Preclinical
NAGS	N-Acetylglutamate Synthetase	237310	Clinical
CPS I	Carbamoyl Phosphate Synthetase	237300	Clinical
Argininosuccinic aciduria	Argininosuccinate Lyase	207900	Clinical
Citrullinemia	Argininosuccinate Synthetase	215700	Clinical
OTC	Ornithine Transcarbamylase	311250	Clinical
Crigler Najjar	Glucuronyl transferase type I	218800	Clinical
Familial Hypercholesterolemia	LDL receptor	143890	Clinical
Refsum Disease	Phytanic acid oxidase deficiency	266500	Clinical
Wilson's Disease	Copper transporter, ATP7B	277900	Preclinical
Primary Hyperoxaluria	Alanine glyoxylate aminotransferase	259900	Clinical
Familial Intrahepatic Cholestasis	Byler disease; bile transporters	211600	Preclinical/Clinical
Glycogen Storage Disease	GSD 1a, 1b	232200, 602671	Clinical

Legend: Abbreviations employed: LDL, low-density lipoprotein; ATP, adenosine-5'-triphosphate; OMIM, online Mendelian Inheritance in Man (www.ncbi.nlm.nih.gov); NAGS, N-acetylglutamate synthetase; CPS I, carbamoyl phosphate synthetase I; OTC, ornithine transcarbamylase; see text for additional abbreviations