

Liver cell transplantation for the treatment of inborn errors of metabolism

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Summary Over the last 15 years, liver cell transplantation (LCT) has developed from an experimental laboratory technique to a potentially life-saving therapeutic option. Because of its minimally invasive nature, the method is especially attractive for (small) children. In children with liver-based inborn errors of metabolism, this transfer of enzyme activity can be regarded as a gene therapy, which can be installed independently and additionally to conservative treatment concepts. To date 14 children with inherited metabolic diseases have undergone LCT in various centres. Although individual results are encouraging, different treatment protocols, difficulties in the objective assessment of function of the transplant, and finally the lack of a controlled study make it difficult to judge the overall significance of LCT in the treatment of metabolic diseases and call for collaborative clinical research.

Abbreviations

LCT	liver cell transplantation
OLT	orthotopic liver transplantation
UCD	neonatal urea cycle defect

CNS	Crigler–Najjar syndrome
GSDI	Glycogen storage disease I
PKU	phenylketonuria
APOLT	auxiliary partial OLT

Introduction

The concept of liver cell transplantation (LCT) as a new therapeutic concept for inborn errors of metabolism was introduced into clinical practice in 1994, two years after the first application in human patients (Mito et al 1992) with liver cirrhosis. Grossman and Raper treated five patients with homozygous familial hypercholesterolaemia by transplantation of genetically modified autologous hepatocytes (Grossman et al 1994). 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 (Strom et al 1997). Since then, a total of 15 patients with hepatic-based metabolic diseases have undergone allogeneic LCT (Table 1) with in general encouraging results that hold out a prospect for the development of new clinical concepts.

Indications

As in every innovative therapy, suitable patients and diseases have to be carefully selected, sometimes through trial and error. It seems reasonable to start with diseases which have rather inadequate therapeutic options and a poor prognosis. Although less

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Table 1 Overview of human LCT in metabolic diseases

Patient	Diagnosis	Age	Vascular access	Applications	Cell dose [10 ⁹ /kg]	Outcome
UCD 1	OTC deficiency (Strom et al 1997)	5 years	Transhepatic	2	0.09	Died
UCD 2	OTC deficiency (Horslen et al 2003)	Neonate	Umbilical vein	13	3.14	OLT
UCD 3	OTC deficiency (Mitry et al 2004)	Neonate	Umbilical vein	1	0.21	OLT
UCD 4	ASL deficiency (Stephene et al 2006)	3 years	Mesenteric vein	11	0.36	OLT
UCD 5	OTC deficiency (Stephene et al 2005)	1 year	Mesenteric vein	10	0.24	OLT
UCD 6	Citrullinaemia (Fisher and Strom 2006)	2 years	N.A.	2	0.25	OLT
CNS 1	CNS I (Fox et al 1998)	10 years	Transhepatic	1	0.20	N.A.
CNS 2	CNS I (Ambrosino et al 2005)	9 years	Transhepatic	1	0.25	OLT
CNS 3	CNS I (Hughes et al 2005)	3 years	Mesenteric vein	10	0.29	N.A.
CNS 4	CNS I (Stephene et al 2007)	9 months	Mesenteric vein	14	0.33	N.A.
CNS 5	CNS I (Allen et al 2005)	8 years	Transhepatic	1	0.06	N.A.
GSD 1	GSD Ia (Muraca et al 2002a)	47 years	Mesenteric vein	1	0.04	N.A.
GSD 2	GSD Ib (Lee et al 2007)	18 years	Transhepatic	3	0.10	N.A.
Refsum	Refsum disease (Sokal et al 2003)	4 years	Mesenteric vein	6	0.13	N.A.
FVIIID 1	Factor VII deficiency (Dhawan et al 2004)	3 months	Mesenteric vein	3	0.16	OLT
FVIIID 2	Factor VII deficiency (Dhawan et al 2004)	3 years	Mesenteric vein	5	0.11	OLT

UCD, urea cycle disorder; CNS, Crigler–Najjar syndrome; GSD, glycogen storage disease, FVIIID, factor VII deficiency; OLT, orthotopic liver transplantation; NA, not available

invasive than orthotopic liver transplantation (OLT), LCT is still an experimental and invasive method, and the expected benefit for the patient must outweigh the known and unknown risks of the new therapy. Therefore, a child with a neonatal urea cycle defect (UCD) was chosen as the first patient. Since the introduction of alternative pathway medication to enhance nitrogen excretion almost thirty years ago, the survival of UCD patients has increased considerably (Enns et al 2007). However, a mortality rate of 85% at the age of 10 years was found in a large European cohort, even though the patients received optimal pharmacological and dietary treatment (Bachmann 2003). Moreover, neurological sequelae such as mental retardation, cerebral palsy, cortical blindness or epilepsy are common among surviving patients, especially those with neonatal onset of the disease (Gropman and Batshaw 2004).

Similar considerations can be made for Crigler–Najjar syndrome (CNS). CNS type I is caused by an absent UGT-glucuronosyl-transferase leading to unconjugated hyperbilirubinaemia and kernicterus. It was uniformly lethal at the time of its first description in 1952. Phototherapy as in neonatal hyperbilirubinaemia can substantially reduce bilirubin levels in CNS I patients. However, phototherapy becomes less effective as the skin thickens with age. Apart from direct side-effects like hyperkeratosis, daily phototherapy of 12 h or more means a profound psychosocial handicap for affected children and especially adolescents (Bosma 2003). Moreover, severe neurological impairment and

death still occur. In a world registry of 57 patients, 4 of the 36 patients who were conservatively treated had severe neurological handicaps, and another 4 died (van der Veere et al 1996).

Glycogen storage disease I (GSDI) and phenylketonuria (PKU) are common inborn errors of metabolism that are usually not considered for OLT. However, GSD I patients can have severe problems in following the strict diet and are always at risk for episodes of hypoglycaemia, seizures and possible brain damage. Moreover, long-term complications such as gout, growth failure and hepatic adenomas with the risk of malignant transformations are related to the quality of metabolic control through therapy (Iyer et al 2007). In PKU, therapeutic success is determined by cognitive outcome rather than mortality. Again dietary compliance can be problematic in individual patients and overall IQ (Burgard et al 1996) and other neuropsychological functions such as attention, processing speed, memory and learning (Anderson et al 2007) may be suboptimal even in early-treated PKU patients. Along these lines, LCT could be conceptually considered to improve the long-term outcome of inborn errors of metabolism that seem inappropriate for OLT.

However, liver or liver cell transplantation can only compensate for hepatic enzyme deficiencies. If the underlying enzyme defect is not located exclusively in the liver, such tissue replacement strategies may not work. This applies for some organic acidurias. About 30 children with methylmalonic aciduria and propionic

aciduria have undergone OLT so far. Because of considerable extrahepatic enzyme expression, there was still high pathology and even mortality after OLT. In particular, neurological damage has occurred even years after transplantation (Chakrapani et al 2002; Leonard et al 2001). In UCD, enzyme activity outside the liver, i.e. in the gut and in the kidneys, is much lower and usually does not cause complications after OLT.

In principle, positive results of OLT are mandatory for any disease that is considered for LCT, and we will try to summarize this aspect in the following. The largest experience exists for UCD. During the last 10 years, there has been a paradigm shift and OLT has become by far the best therapeutic option for UCD, with long-term survival rates of about 90% (Morioka et al 2005). Also in CNS I, OLT or auxiliary partial OLT (APOLT) is gaining increasing significance. As discussed earlier, in this disease the risk of neurological damage and the daily need for hours of phototherapy can be eliminated by liver transplantation (van der Veere et al 1996). OLT is rarely performed in GSD I. However, there seems to be a certain subset of patients with limited response to conservative therapy; and the results of OLT in these children are again excellent (Iyer et al 2007).

Since only small amounts of liver cells can be transferred by LCT, not every condition that can be treated with OLT is also suitable for LCT. For example, OLT works well in primary hyperoxaluria, but as about 90% of the donor liver tissue has to be replaced to eliminate the nephrotoxic metabolite, LCT is not an option for this disease. APOLT has been successfully performed in UCD, CNS I and GSD I patients. This is no surprise, as about one-third of the donor liver is replaced in APOLT, which makes the recipient comparable to a healthy heterozygous

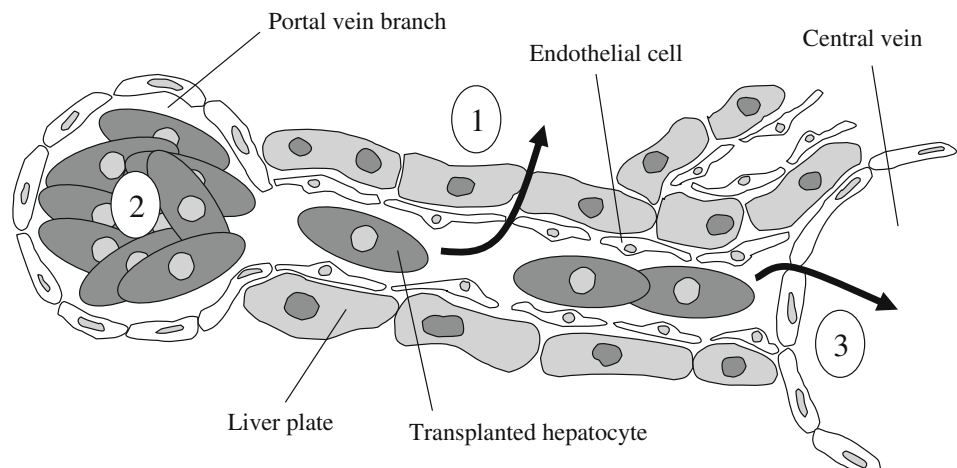
carrier. By using heterozygous parents as living donors, the threshold of transplanted enzyme activity is considerably lowered, but is often still sufficient. A Japanese girl with citrullinaemia received a left lateral graft from her heterozygous mother. A calculated transferred enzyme activity of 8% was sufficient to correct the phenotype (Ban et al 2001). It is frequently speculated in the literature that about 5–10% of enzyme activity is needed to compensate for most inborn errors of metabolism, but exact figures are lacking.

Side-effects and safety considerations

The safety of liver cell transfusion is closely related to the amount of cells applied. If a certain threshold is reached, shunting of the transplanted cells into extrahepatic organs may occur and the risk of portal vein thrombosis is increased (Fig. 1). Although it has been shown in preclinical studies that these side-effects are dose-related, the exact safety margin varies between different animal models. In a canine model of LCT, deaths have been reported at single doses of 1.5% of total liver cell count (Kay et al 1992), whereas applications of 4% of the total liver cell count were well tolerated in a rat model (Rajvanshi et al 1996b). The threshold for pulmonary shunting also varies between different species (Kocken et al 1997; Muraca et al 2002b; Rajvanshi et al 1999; Schneider et al 2003), and in the vast majority of animal studies it was never observed. Interestingly, it has been shown in a rat model that hepatocytes are rapidly destroyed after 24 h, should they eventually reach the pulmonary circulation (Rajvanshi et al 1999).

The risk of the development of portal vein thrombosis depends on the impairment of portal vein flow, which is in turn correlated to the amount of transplanted cells.

Fig. 1 Potential risks of hepatocyte application. Transplanted hepatocytes enter the liver plate via the fenestrae of the sinusoid epithelial cells (1). Large amounts of cells infused into the portal vein at once may cause portal vein obstruction and subsequent thrombosis (2). If the capacity of the sinusoid is grossly exceeded, transplanted cells may enter the systemic circulation and cause pulmonary embolism (3)



It has been shown in a pig model that the increase of portal vein pressure during application is directly related to the cell dose (Muraca et al 2002b). However, in animal studies it was shown that the impairment of portal vein flow is only transient, with normalization within 1–2 h after infusion (Attaran et al 2004; Kocken et al 1996; Schneider et al 2003). In some of these studies, transient increases in liver enzymes have also been reported. These changes do not seem to be solely related to the transfused cells, because they can also be demonstrated after portal infusion of albumin macroaggregates (Schneider et al 2003). It can be speculated that such an ischaemic response may even facilitate cell engraftment by disrupting the endothelial membrane of the liver sinusoids, as regional transient ischaemia has been shown to improve transplant success in the animal model (Attaran et al 2004).

In human LCT, these potentially life-threatening side-effects have been very rare. At present, about 80 therapeutic attempts in humans have been published (Fisher and Strom 2006). Clinically relevant cell shunting into the pulmonary circulation has so far only been described in one patient with liver failure and pre-existing portosystemic shunts due to liver cirrhosis (Bilir et al 2000). Portal vein thrombosis has only been described in a single case so far; furthermore, it developed in a patient with graft failure after liver transplantation and a pre-existing stenosis of the portal vein anastomosis (Baccarani et al 2005). In a recent review, thrombosis of a mesenteric vein after LCT was mentioned in one patient, but no further details are available (Fisher and Strom 2006). Thus, relevant side-effects in clinical applications have only been observed so far in adults with co-existing diseases, especially structural pathology of the liver. Two precautions are crucial to keep the rate of side-effects low: serial applications and thorough monitoring of the cell infusion.

The concept of serial cell transplantation has been developed in parallel with the first clinical applications of LCT. In a rat model it was shown that serial transplantations increase the number of engrafting cells without increasing the rate of complications (Rajvanshi et al 1996a). In other animal studies it was confirmed that fractionated application of cells increases the safety of the procedure by minimizing the risk of portal vein obstruction and shunting of the transplanted cells into the pulmonary circulation (Attaran et al 2004; Kocken et al 1996; Schneider et al 2003). Serial transplantation (2–14 individual applications) has been used in human LCT since 1997 for cases of acute liver failure (Bilir et al 2000; Schneider et al 2006; Soriano et al 2001) and, more frequently, metabolic diseases (Dhawan et al 2004; Horslen et al

2003; Lee et al 2007; Sokal et al 2003; Stephenne et al 2005, 2006; Strom et al 1997). Suitable catheters or port devices in the portal vein system allow a time-frame of up to several months for fractionated applications in children with metabolic diseases (Darwish et al 2004).

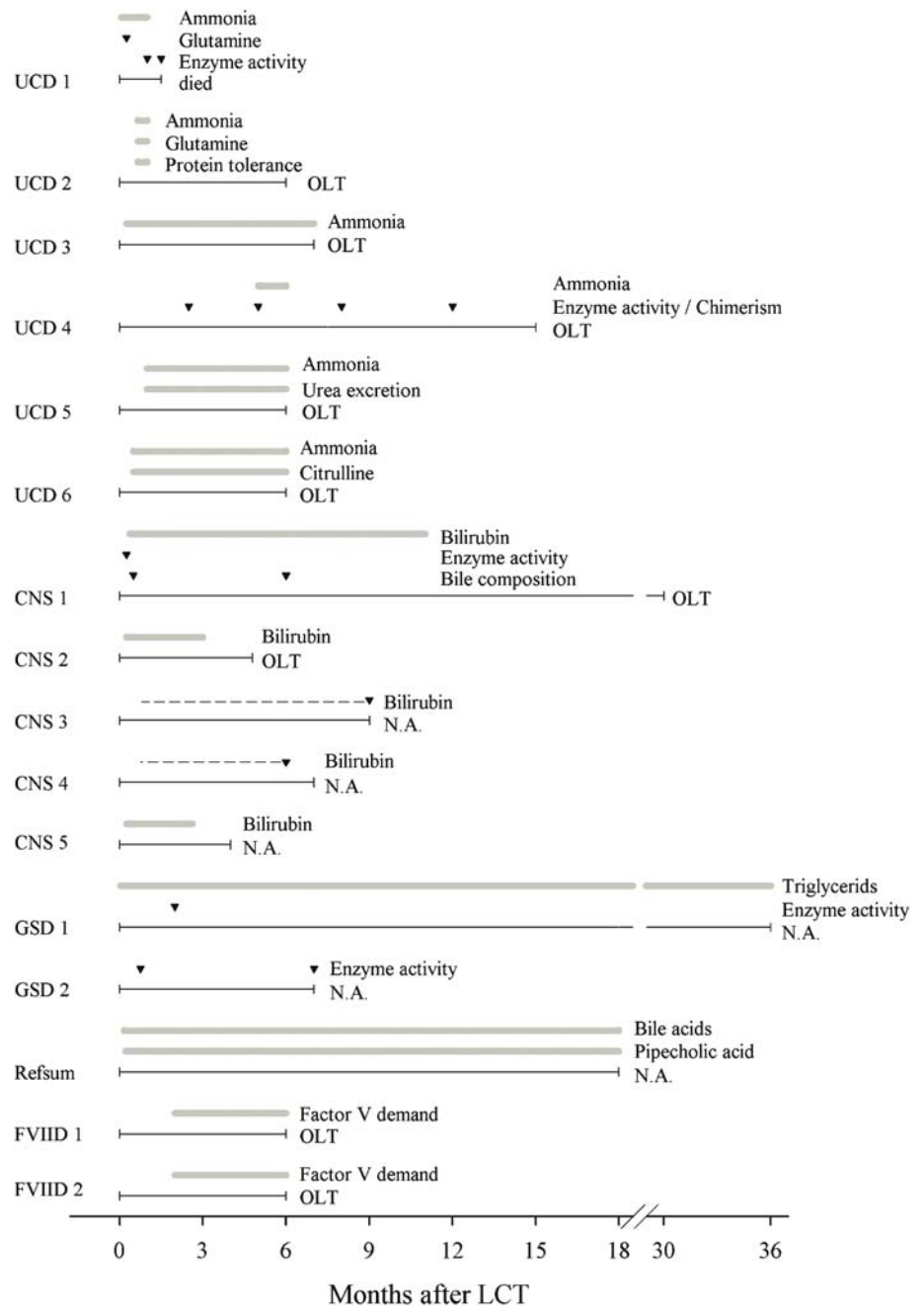
Direct determination of the portal vein pressure is technically feasible in human liver cell application. From animal data, it seems reasonable to pause cell application if the portal vein pressure increases by more than 50% of the pre-application values (Kocken et al 1996; Muraca et al 2002b). However, if measurement of portal vein pressure is reported in human applications, only pre- and post-application values are given. A more continuous observation of portal vein flow can be achieved by Doppler ultrasound examination of the portal vein during cell application. Maximal flow velocity and portal vein flow have been investigated in the rabbit (Schneider et al 2003), and the same group demonstrated the feasibility of this technique in an adult with acute liver failure (Schneider et al 2006).

The story so far

At the present time, published data are available of 14 patients who have been treated with LCT for inborn errors of metabolism (Table 1). Two additional cases of children with inherited factor VII deficiency should be included; although not a metabolic disease, this liver-based enzyme deficiency is largely comparable to the metabolic diseases treated with LCT. Apart from the two adult patients with GSD I, LCT for metabolic diseases has been a paediatric issue so far. It has even been used in the neonatal period in individual cases.

Key data of the 16 patients are given in Table 1 and Fig. 2. The first human LCT for an inborn error of metabolism was done 1997 by the Pittsburgh group (UCD 1). A 5-year-old boy with OTC deficiency initially showed marked improvement in his laboratory parameters. Pathological levels of ammonia and glutamine normalized within 48 h. However, he experienced a severe metabolic decompensation 4 weeks after the transplantation following a protocol liver biopsy. He subsequently died after 2 weeks from pneumonia (Strom et al 1997). A male neonate with a prenatally established diagnosis of OTC deficiency (UCD 2) was treated with LCT via an umbilical vein catheter immediately after birth. Laboratory parameters as well as protein intolerance improved only slightly. The authors speculate that insufficient immunosuppression might have caused rejection of the

Fig. 2 Assessment of transplantation success in human trials of LCT. Observation periods are given by the thin bottom line of each case. Grey lines represent repeated monitoring of key parameters over a period of time, triangles single investigations. UCD, urea cycle disorder; CNS, Crigler–Najjar syndrome; GSD, glycogen storage disease; FVIID, factor VII deficiency; N.A., further outcome information not available



transplanted cells (Horslen et al 2003). This neonatal setting was repeated more successfully in another boy with prenatally diagnosed OTC (UCD 3) by the London group. After transplantation, he even tolerated a normal supply of protein, and no metabolic crises occurred. Because there were uncertainties about the long-term stability of restored enzyme activity after LCT, and because of severe social problems complicating the outpatient management (A. Dhawan, personal communication), he received a liver graft after 7 months and is doing well (Mitry et al 2004). A 3-year-

old girl with argininosuccinate lyase (ASL) deficiency (UCD 4) was treated in Brussels (Stephenne et al 2006). The amount of transplanted cells from a male donor was estimated to be about 5% from multiple liver biopsies using a FISH technique to detect Y-chromosomes. A 14-month-old boy with OTC deficiency (UCD 5) was already listed for liver transplantation. LCT was offered to stabilize the child until an organ was available. The patient's ammonia levels decreased while urea production increased after the cell transplantation, which was repeated after

5 months. Four weeks after the last transfusion, a suitable organ was available for liver transplantation. This was the first patient who received exclusively cryopreserved cells (Stephenne et al 2005). Information about a 2-year-old patient with citrullinaemia (UCD 6) treated in Seoul is sparse. Apparently, laboratory parameters improved over a period of 6 months following LCT before the patient underwent OLT (Fisher and Strom 2006).

A 10-year-old girl (CNS 1) with Crigler–Najjar syndrome type I is generally regarded as the first metabolic patient with a long-term improvement after hepatocyte transplantation. Following a single liver cell transfusion, bilirubin levels decreased about 50%, thus allowing a significant reduction of daily phototherapy. After 2 1/2 years, the patient and her family objected to another hepatocyte transplantation, and she received a normal liver graft (Fox et al 1998). A second child (CNS 2) with Crigler–Najjar syndrome type I, a 9-year-old boy, was treated in Italy. Although the dose of transplanted cells was comparable to that of the first CNS I patient, the initial reduction in serum bilirubin subsided after only 3 months. OLT was performed successfully 2 months later (Ambrosino et al 2005). Few details are available for the remaining three CNS I patients. In a 3-year-old girl (CNS 3), beneficial effects lasted at least 9 months and were still present at the time of publication (Hughes et al 2005), whereas they had already disappeared in two other girls (CNS 4 and CNS 5) after 6 and 3 months, respectively (Allen et al 2005; Stephenne et al 2007). Whether the last three patients underwent OLT in the meantime is not known.

Two adult patients with glycogen storage disease types Ia and Ib, respectively, have been treated with LCT so far. The fasting tolerance of a 47-year-old woman (GSD 1) improved after LCT, and triglyceride levels decreased markedly. The metabolic improvement lasted at least 3 years (time of last publication), which is the longest period of beneficial effects after LCT described so far (Burlina 2004; Muraca et al 2002a). The other patient (GSD 2) was an 18-year-old man with glycogen storage disease type Ib and multiple hepatic adenomas. He showed a good initial response, fasting tolerance significantly improved, raised lactate levels normalized, and he was clinically well during an observation period of 7 months (Lee et al 2007). A 4-year-old girl with a peroxisomal metabolic defect (infantile Refsum disease) was the first patient of the Brussels LCT programme. Because of severe neurological impairment, liver transplantation was not considered. Following LCT, the key biochemical metabolite decreased substantially, reaching 61%

of the original value after 18 months. Surprisingly, her overall condition also showed a marked improvement over that period; she gained weight and started to stand and walk (Sokal et al 2003). Finally, LCT was performed in an attempt to cure factor VII deficiency in two brothers (3 months and 2 years). Although an initial decline in the substitution of factor VII demand was documented, the effect was only partial and resolved after 25–30 weeks. Thus, both children underwent liver transplantation (Dhawan et al 2004).

Open questions

The key issue in LCT at the present time is the detection and quantification of transplant success, i.e. restoration of activity of the deficient enzyme. There is still a discrepancy between near-total liver repopulation in animal experiments and the somewhat limited effects in humans so far. All very successful animal experiments have used some kind of preconditioning of the host liver to give the transplanted cells a repopulation advantage. Commonly used methods for such preconditioning are cytotoxic agents, ischaemia, irradiation or partial hepatectomy, all of which induce a proliferative stimulus for the transplanted cells. None of this appears possible in children. On the other hand, in animal experiments transplanted hepatocytes can be labelled in various ways to be detected later in histological sections. The host organ may be removed after LCT, the cells isolated by collagenase perfusion as the donor liver before, and the donor cells quantified in the resulting cell suspension (Koenig et al 2005). In human LCT trials, liver tissue for direct detection of the cells must be gained from liver biopsies. In two cases of sex-mismatched LCT, male cells transplanted into female livers could be detected and quantified by means of PCR (Wang et al 2002) or FISH analysis (Stephenne et al 2006). In another study, genetic markers of the donor were used to identify transplanted cells by means of tandem-short-repeats analysis (Mas et al 2004). Unfortunately, these methods have a potentially huge sampling error because of the irregular patterns of distribution of the transplanted cells and the small sampling volume. For metabolic diseases, functional testing using stable-isotope measurements is a tempting alternative. For example, promising results of quantifying the flux through the urea cycle in homozygous and heterozygous UCD patients have been published (Lee et al 2000). Further work on this topic will hopefully allow serial monitoring with reliable quantitative results in human LCT for metabolic diseases.

For the time being, the judgement of transplantation success in human LCT relies on clinical evaluation and conventional laboratory data. Progress in human studies is hampered by three obstacles: (1) *In animal experiments larger amounts of cells can be transplanted and a higher percentage can be engrafted.* With methods of preconditioning the host liver prior to LCT, repopulation can be considerably enhanced. As a consequence, differences in the formation of substrates reflecting gains in enzyme activity are much more pronounced in the animal but may be less significant or may even be missed in human patients treated with LCT. (2) *Some metabolic diseases exert their pathological consequences not only in the liver and are integrated into complex pathophysiological sequences.* In CNS I patients, the decline in plasma bilirubin can be easily quantified, whereas ammonia and glutamine formation in UCD are dependent not only on enzyme activity but critically on protein catabolism and environmental factors. Furthermore, transporters and specific functions of the urea cycle are also relevant and expressed in extrahepatic organs, e.g. kidney and brain. (3) *Clinical and laboratory data may be misleading.* In UCD (and to a lesser extent in GSD) patients, long periods of metabolic stability can be achieved by conservative treatment alone; therefore the specific effect of the transplanted cells may remain unclear.

Another major open question is the amount of cells that is needed to achieve substantial metabolic improvement. Of course, this issue is closely related to the considerations above about the quantification of transplant success. To draw conclusions from animal experiments, only studies that did not use preconditioning of the host liver prior to LCT can be evaluated. For this purpose, the best model is the Watanabe hereditary hypercholesterolaemia (WHHL) rabbit. A few studies in this model meet important criteria that are required in human LCT: allogeneic transplantation, immunosuppression, and intraportal application (single or fractionated applications). In these studies, a considerable decrease in serum cholesterol could be demonstrated after transplantation of 2–4% of the recipient's calculated total hepatocyte count (Attaran et al 2004; Gunsalus et al 1997; Wiederkehr et al 1990; Wilson et al 1988). Similar considerations have been made in cases of human LCT. For the first successful LCT in a metabolic disease (CNS I), a total cell count of 280×10^9 hepatocytes was assumed for an adult liver (Fox et al 1998). Given an average weight of 70 kg, the total hepatocyte count would be 4×10^9 per kg body weight. Although this calculation neglects the sigmoid growth curves in infancy and childhood, it has been the basis for dose calculations ever since. Thus, a dose

targeted at replenishing 5% of the total liver cell count corresponds to 0.2×10^9 cells per kg. In most published cases of LCT in metabolic diseases, cell doses of 0.1×10^9 to 0.35×10^9 per kg, which is 2.5–8.25% of the calculated total hepatocyte count, have been used. It is known from animal data that only about one-third of the transplanted cells engraft permanently in the liver (Gupta et al 1999). Although never studied in humans, it is reasonable that similar conditions also apply for human LCT. Thus, only about 1–3% of the calculated total hepatocyte count would have been transferred in the reported cases. It seems unlikely that the observed beneficial effects of LCT can be attributed to such low amounts of transferred enzyme activity. Therefore, the transplanted cells must have proliferated to some extent, even without preconditioning of the host liver. Alternatively, upregulation of the transferred metabolic functions may play an important role. Such mechanisms have been observed under other conditions for urea cycle enzymes (Lee et al 2003; Tygstrup et al 1995) but have not been investigated so far in the context of LCT.

Conclusions

Liver cell transplantation is a fascinating new therapeutic concept for the treatment of inborn errors of metabolism. Most of the patients treated so far have experienced metabolic improvement that lasted for months or even years in individual cases. In some conditions such as Crigler–Najjar syndrome, the effects of LCT are quantifiable quite easily, in others such as UCD, the lack of an objective parameter to quantify transplanted enzyme activity or metabolic flux through the urea cycle is still a major problem. As a consequence loss of function of the transplanted cells (i.e. rejection) will be very hard to foresee and to recognize, and OLT has subsequently been performed in most cases (Table 1). On the other hand, LCT is a safe and only slightly invasive technique. It does not carry the risk associated with permanent removal of the liver in OLT. Furthermore, it has the potential of repeated applications over long time intervals. It has been shown before that cryopreserved liver cells may provide metabolic stability in inborn errors of metabolism (Stephene et al 2005). If cryopreserved cells could be developed into a biological drug distributable to metabolic centres within hours, this would be an important prerequisite for the necessary clinical trials. We have to define and prove clear indications for LCT as an additional therapeutic approach in the diseases considered so far such as Crigler–Najjar

syndrome type I and neonatal urea cycle disorders. LCT may then develop further into an option for more 'benign' inborn errors of metabolism such as maple syrup urine disease or PKU.

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