Abstract book

MEDICAL PROGRESS AND PITFALLS OF THE TREATMENT OF INBORN ERRORS INVOLVING THE LIVER

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In the early years because of the high risk of mortality and morbidity, liver transplantation was only used for those that were terminally ill. As the outlook for liver transplantation in childhood has improved so the indications for transplantation for metabolic disorders in childhood have expanded (1).

Liver transplantation is used to treat inborn errors for two major indications; firstly because of irreversible liver disease, usually cirrhosis, and secondly for enzyme replacement. The management may be made more complicated by the involvement of other organs by the metabolic disease.

The indications for transplantation for isolated liver failure are the same as those for any other cause. The most frequent disorders are progressive familial intrahepatic cholestasis (PFIC), Wilson's disease, α1- antitrypsin deficiency and tyrosinaemia type 1. In glycogen storage disease (GSD) IV, cystic fibrosis, respiratory chain disorders, and cholesterol ester storage disease liver may be also diseased but other organs are often involved (heart, lung, etc) and may complicate transplantation.

Liver transplantation for enzyme replacement has been done for many disorders. These include Crigler-Najjar, hyperoxaluria, GSD type 1, maple syrup urine disease and urea cycle disorders (most often carbamyl phosphate synthetase and ornithine transcarbamylase deficiencies). The disorder frequently involves other organs, for example in methylmalonic acidaemia, respiratory chain disorders and hyperoxaluria. For this reason in hyperoxaluria and methylmalonic acidaemia the liver transplantation is often combined with renal transplantation because of end stage renal failure (2). Early transplantation may be valuable in some disorders to prevent inevitable complications (for example – homozygous familial hypercholesterolaemia) but the indications for many disorders are not clearly defined. These may include poor prognosis despite optimal therapy, risk of additional complications, difficulties with treatment resulting in unstable metabolic control and poor quality of life.

Whilst intellectual function may improve with liver transplantation (3) liver transplantation is generally not indicated in those disorders in which there is already severe systemic complications (systemic oxalosis), severe neurological damage or in which such complications are inevitable such as Alpers disease (4) and Niemann- Pick type C. However the underlying diagnosis may not be apparent when the patients first present, particularly if they present with acute liver failure. However with advances in molecular genetics it may become possible to screen for some disorders, such as Alpers syndrome.

When considering liver transplantation for systemic disorders it is important to understand the condition fully as serious unexpected complications may develop. In methylmalonic acidaemia it was widely thought that liver transplantation would effectively cure the disorder by reducing circulating metabolite concentrations. However it is now recognised that these patients are at risk of neurological complications, particularly around the time of operation but even many years after transplantation (5).

With increasing use of living related donors from a close family relative, the problem of suitability of carriers of metabolic disorders arises. Whilst there does not appear to be a problem with heterozygotes of recessive disorders, female carriers of X-linked conditions, such as ornithine transcarbamylase deficiency, will need very careful assessment.

The enzyme activity necessary to correct the metabolic abnormalities in some disorders is small so that the disorder may be treated effectively by the replacement of only some liver tissue (for example Crigler-Najjar). In which case hepatocyte transplantation or partial auxiliary liver concentration may be all that is necessary but in both these disorders monitoring the viability of the donor tissue is difficult. Partial auxiliary liver transplantation is technically difficult (6) and relatively few patients have had this procedure worldwide. Hepatocyte transplant may be used to bridge a gap before liver transplantation (7).

Liver transplantation for inborn errors is now an established procedure. Whilst the indications for liver failure are clearly defined the timing and indications for the treatment of inborn errors for enzyme replacement therapy are much less so. The outcome of all patients being considered for transplantation needs to be documented carefully so that guidelines can be developed.

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LIVER TRANSPLANTATION FOR INHERITED METABOLIC DISEASE

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Abstract

Liver Transplantation is indicated for children with inherited metabolic disease because of a primary hepatic enzyme deficiency, which leads to liver failure and/or hepatic cancer, or severe extra hepatic disease. The selection and timing depends on the rate of progression of disease, the success of medical therapy and the extent of reversible extra hepatic disease. Transplant techniques include orthotopic, living related and auxiliary liver transplantation or combined liver and kidney transplantation.

Liver transplantation effectively treats structural metabolic hepatic disease with a phenonotypic and functional cure in alpha1 antitrypsin deficiency, Wilson's disease, PFIC and neonatal haemochromatosis. There is only a partial cure in Tyrosinaemia Type 1 as abnormal metabolites are still produced by the kidney, but a complete cure in primary oxalosis and urea cycle defects. Outcome for organic acidaemias remains poor due to technical and metabolic challenges.

The advent of successful immunosuppression and improved survival rates for liver transplantation has extended the indications to the treatment of inherited metabolic disease.

The aim of liver transplantation in this situation is to cure the inherited metabolic defect both functionally and phenotypically. It is indicated in children with inborn errors of metabolism due to primary hepatic enzyme deficiency which either leads to liver disease and hepatic cancer or to severe extra hepatic disease. In some cases liver transplantation may be required in combination with kidney, heart or bone marrow transplantation.

Inherited metabolic disorders leading to hepatic disease

1. Specific hepatic enzyme deficiencies leading to acute or chronic liver failure and/or development of hepatic cancer : - alpha-1-antitrypsin deficiency, tyrosinaemia type 1, glycogen storage disease type 4.
2. Liver failure secondary to other hepatic enzyme defect : - Wilson's disease, PFIC, neonatal haemochromatosis.
3. Hepatic enzyme deficiency leading to acquired liver disease. Factor VIII and IX deficiency with subsequent viral hepatitis.

Inherited metabolic disorders, leading to extra hepatic disease

Crigler-Najjar Type I, primary oxalosis, familial hypercholesterolaemia, urea cycle defects and organic academia.

Patient selection and timing of transplantation

The selection of patients and timing of transplantation depends on the enzyme deficiency, the extent of hepatic or extra hepatic disease, the quality of life and outcome and the availability of medical treatment.

Contra-indications for liver transplantation for metabolic disease

The current contra-indications include those children with severe extra hepatic disease which will not reverse post transplantation (eg. severe systemic oxalosis, mitochondrial disorders with associated muscle and neurological degeneration).

Operative techniques

The successful development of reduction hepatectomy, split liver transplantation and living related transplantation has extended the age and size range of transplantation to young babies with comparable results to whole graft transplantation.

As many children with metabolic disease have morphologically normal livers, auxiliary liver transplantation is an option. In this technique the left lateral segments of the patient's liver are replaced with the same segments from a donor liver. Despite initial technical difficulties this technique has proved successful for patients with Crigler Najjar Type I and mild propionic acidemia. Living related liver transplantation is also effective in certain disorders.

Post-operative management

The post operative management of children with inherited metabolic disease is similar to other indications. Most centres prevent graft rejection with either Prednisolone, Azathioprine, Cyclosporin or Tacrolimus steroids and reduce the number of drugs and dosage with time. The range of surgical and medical complications is similar for those children transplanted for liver failure. The commonest complications are rejection 50%, sepsis 75%, arterial and venous thrombosis 10-20% and biliary complications 10-20%.

Correction of the metabolic defect

In alpha-1-antitrypsin deficiency, PFIC and Wilson's disease there will be both phenotypic and functional cure. In tyrosinaemia type 1 liver transplantation corrects the hepatic enzyme deficiency and prevents the development of liver cancer although the kidney continues to produce toxic metabolites. In Crigley-Najjar Type I, urea cycle defects and primary oxalosis, the metabolic defect is completely corrected. In the organic acidemias the metabolic defect is widespread throughout body tissue but liver replacement provides sufficient hepatic enzyme to prevent metabolic acidosis under normal conditions. However, these children are still at risk of mild metabolic acidosis during intercurrent infections and outcome overall remains poor.

Survival and quality of life

One year survival for liver transplantation in children ranges from 90-95% with 80% surviving 5 years. A number of studies suggest that children transplanted for inherited metabolic disease have improved survival with one year survival rates of 95% and 4 year survival rates of 88%.

Long term problems are related to immunosuppression, nephrotoxicity, an increase in viral infections and the potential to develop lymphoproliferative disease. Nevertheless, survivors have dramatic improvement in quality of life as their strict dietary regimes and difficult metabolic control become redundant.

The future development of molecular genetics, effective gene therapy and hepatocyte or stem cell therapy may displace liver transplantation as treatment of these disorders in the future.

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MEDICAL VERSUS SURGICAL MANAGEMENT OF PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS - PFIC

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Pharmacological methods used so far for treating PFIC have not proved clear efficacy. Clinical and biochemical improvement was observed only in a few children treated with ursodeoxycholic acid (UDCA).

In the last decade partial external biliary diversion (PEBD) became standard procedure performed on PFIC children with no response to medical treatment. There is still a group of patients who cannot benefit from this procedure because of technical conditions (earlier performed cholecystectomy) or postoperative complications like dyselectrolytaemia due

to the excessive amount of bile. In 1998 Holland *et al*. described ileal bypass (IB) as promising in PFIC children after cholecystectomy. The only treatment for children with PFIC and hepatic cirrhosis remains liver transplantation.

The aim of our study was a retrospective evaluation of different methods of treatment in children with PFIC: pharmacological therapy with phenobarbital, cholestyramine and UDCA, as well as surgical methods, PEBD, IB, as alternative methods to liver transplantation.

Material and methods

Retrospective review of records of all 59 (36 boys, 23 girls) children with PFIC was undertaken. Provisional diagnosis was established based on anamnesis, clinical symptoms (jaundice, pruritus, hepatomegaly) and laboratory findings (all children had **low** GGTP activity and high concentration of bile acids in serum). Other causes of intrahepatic cholestasis were ruled out. Diagnosis was confirmed by genetic study in 32 children (in three children PFIC type 1 was diagnosed, in 29 children PFIC type 2). Immediately after diagnosis of PFIC pharmacological therapy was started in all patients. This was the only treatment used before 1990, when liver transplant program was initiated in Children's Memorial Health Institute in Warsaw. Since 1996 we have performed PEBD and since 1998, IB.

Results

Treatment with cholestyramine and phenobarbital was ineffective. Total relief of clinical symptoms and normalisation of biochemical results were seen in 11 (22%) children out of 50 treated with UDCA. Nine patients (15%) treated only conservatively died. Liver transplantation (LTx) was performed in 13 children (including 5 patients after PEBD). Two of them died in the early posttransplant period due to postoperative complications. Eleven children after LTx are alive, with a follow-up between 2 and 11 years after transplantation. PEBD was undertaken in 28 patients (1 was converted from IB). The result of the therapy was very good in 21 (75%) pts.

Since 1998 IB has been performed on 7 children : in 4 children as the first operation – 3 were converted from PEBD (in 2 cases due to the postoperative complications of stoma (dyselectrolitemia), in 1 case due to psychological – aesthetic reasons). IB was ineffective in 5 children, one of them had later PEBD, one is now on waiting list for liver transplantation, 3 have still elevated bile acids despite UDCA therapy.

Conclusions

Medical treatment was ineffective in majority of patients with PFIC. Surgical treatment gave better results in these children. The best results were observed after partial external biliary diversion.

IN VITRO TISSUE EXPANSION PERSPECTIVES

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Besides orthotopic liver transplantation, therapies currently developed to treat liver diseases include transplantation of isolated mature adult hepatocytes by their infusion into blood stream, implantation of liver tissue substitutes and perfusion of blood through an extracorporeal bioartificial device containing liver cells.

With respect to liver cell transplantation (LCT), the success of the procedure closely depends on the viability and the stability of liver-specific functions of the infused liver cells. The number of needed hepatocytes is also another limiting factor because of donor organ shortage.

Hence, several attempts are investigating the optimal appropriate conditions for the improvement of both quantity and quality of the cells dedicated to LCT.

Primary hepatocytes represent the most common cell type in current liver-directed cell therapies. However, these cells showed a limited proliferation potential in vitro, even under the most optimal culture conditions, and a loss of differentiated functions over a period of days. The analysis of cell fractions obtained after liver isolation demonstrates that diploid hepatocytes are largely associated to the proliferation observed in primary cultures (1,2). Accordingly, the isolation and purification of this cell population may help to characterize their proliferation state in vitro whereas in vivo studies have to evaluate their engraftment potential. Stimulation of hepatocytes proliferation and differentiation can also occur if co-cultured with other cell types as for instance bone marrow stromal cells (3). These data obtained in adherent cell cultures need to be confirmed in cell suspension culture systems such as bioreactors. Indeed, these systems that allow stability of liver-specific functions thanks to a three-dimensional cultures will help to efficiently supply large amounts of hepatocytes.

At the molecular level, it has been clearly demonstrated that telomere length constitutes one of the parameters that regulate hepatocytes proliferation. Hence, several studies are currently focused on inhibiting its attrition and evaluating its effect on in vitro hepatocytes expansion. According to in vivo data showing that upon appropriate conditions, hepatocytes are able to proliferate with no alteration of differentiated functions, immortalization approaches have been

developed. Oncogenic, viral, or chemical agents have been used for overcoming the growth limitations of primary hepatocytes. However, still additional data are mandatory especially regarding safeguards of their clinical use.

Mechanisms of aging have also been investigated in order to characterize the genes involved in conferring growth advantage and prolonging life span of hepatocytes in vitro.

Finally, isolation and characterization of liver stem and progenitor cells is another recent alternative thanks to the development of cell biology techniques. These self-renewing cell types of adult or embryonic origin may represent new therapeutic prospects if full characterization of the behaviour and the biology of these cells in vivo, has been performed.

In conclusion, advances and knowledge of hepatocytes and liver stem biology in vitro will be helpful to determine the optimal conditions for their in vitro expansion or even prolonged life span in order to counteract the limitation of cell supply and to improve the quality of cell suspension for LCT.

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PROGREES AND PIFALLS IN ORGANIC ACIDURIA

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Organic acidurias comprise many various disorders. Methylmalonic and propionic acidurias are the most frequent diseases and the two organic acidurias for which we have the most important view on the long term outcome.

Patients affected with these disorders mostly present in the neonatal period with a neurological distress of the intoxication type with ketoacidosis and mild to moderate hyperammonaemia. As neonates they most often require emergency treatment with supportive care, toxin removal procedures, high energy and free-protein nutrition. The mainstay of the long term management is a low-protein high-energy diet that is most often supplemented with amino acids omitting the propiogenic ones. This diet must be completed with sufficient vitamins, minerals and micronutrients. Feeding problems are extremely common and these patients most often require enteral nutrition to maintain a good nutritional state. Specific additional therapies are usually used. L-carnitine supplementation prevents from deficiency due to acyl-carnitine excretion. Metronidazole reduces propionate production in the gut. Sodium benzoate has been proposed to correct both chronic hyperammonaemia and hyperglycinemia.

Despite improvements in conventional management, the outcome reported in the early nineties was disappointing with high mortality rate in both the neonatal and late onset forms. In addition, these patients had presented various complications all along their courses and had recurrent decompensations requiring multiple hospitalisations (Leonard 1995). At last, developmental outcome reported in the largest series was poor with less than fifty percent of patients with an IQ higher than 80 (Saudubray et al 1999, van'tHoff et all 1999,).

A more recent retrospective study concerning patients born after 1990 describes some improvement (Ogier de Baulny et al 2005). However, the mortality rate remains high with about 20% patients that have died. Half of these deaths have occurred during the neonatal period. The other half has occurred before the age of ten years.

Independently, forty per cent of the whole population has presented some kind of complications. Renal impairment of various degrees is present in MMA patients as early as 6 years of age. Pancreatitis has been responsible for a late death in a MMA patient. Cardiomyopathy has been developed in 2 PA patients. Neurological complications are dominated by extrapyramidal signs with basal ganglia involvement (6 MMA and 2 PA). Psychiatric disorders with autistic signs, optic atrophy, neurosensorial deafness, and myopathy are other late complications.

The developmental outcome may have improved. However, among patients older than 4 years of age, 40% MMA patients and 60% PA patients have low IQ and require special education.

In summary, it appears that there is some improvement in survival and in developmental outcome. The price to pay is a higher morbidity with, for some patients, a very poor quality of life.

Facing these disappointing results, transplantation has appeared few years ago a real hope (Schlenzig et al 1995, Van'tHoff et al 1999, Leonard et al 2001). However, several reports on liver in both the MMA and PA patients, renal or combined liver and renal transplantation in MMA patients show that the incomplete metabolic correction is responsible for late acute or chronic neurological deterioration and that liver transplantation does not prevent from renal involvement in MMA patients (van Calcar et al 1998, van'tHoff et al 1998, Lubrano et al 2001, Nyhan et al 2002, Chakrapani et al 2002, Burlina et al 2003). In addition, mortality rate (27%) is not negligible. However, survivors are reported with

better quality of life (Goyens et al 1997, Rela et al 1997, Van'tHoff et al 1999, Saudubray et al 1999, Yorifuji et al 2000, Yorifuji et al 2004). These results underline the need for permanent diet and metabolic controls after transplantation.

In conclusion : MMA and PA are severe disorders with poor outcome despite medical attention and some improvement in survival and mental development. They have high morbidity with acute and chronic neurological diseases and various visceral complications incompletely prevented by transplantation. What would be the effect of neonatal screening and prospective treatment liver/ renal transplantation remain open questions.

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REPORT OF LIVER TRANSPLANTATION IN ORGANIC ACIDURIA

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The outcome for childrens suffering from severe and early-onset organic acidurias (OA), such as propionic acidemia (PA) and methylmalonic acidemia (MMA), due to the inherited propionyl-Coa carboxylase and methylmalonyl-CoA mutase deficieny, respectively, remains poor. Despite the conventional treatment with low-protein diet, carnitine and metronidazole supplementation, the course of severely affected patients who survived to the neonatal ketoacidotic coma is characterized by recurrent life-threatening episodes of metabolic acidosis, anorexia, vomiting, failure to thrive, developmental delay, stroke, extrapyramidal signs, pancreatitis, progressive renal disease and cardiomyopathy. Retrospective studies show a 80% mortality within the first 10 years of life . Alternative treatment such as liver transplantation (OLT) has been considered, but this option is not commonly adopted. The goal of OLT in OA is to provide normal enzyme activity at the liver in order to assure a sufficient clearance of toxic organic, deriving mainly from muscular aminoacid catabolism.

Here we report on a male patient with classical cobalamin non-responsive early-onset MMA due to methylmalonyl-CoA mutase deficieny. His urinary MMA excretion was between 6000 and 8000 mmol/mol C and mutase activity measurement in fibroblastes showed a very low enzyme level (< 1 % of normal). The diagnosis was finally confirmed also at the molecular level, as the patient was found to be compound heterozygous at the methylmalonyl-CoA mutase

	Neonatal period At diagnosis	*before-OLT (age: 3 year)*	*A fter OLT (2 yrs follow-up)*
AMM plasma (µmol/L)	ND	716	185
AMM urina (µmol/mmol C)	11.000	3495	800

locus for the two already reported mutations, the G158V and R467X, respectively. Following the neonatal acute onset, he did not show major problems during the first year of life. However, since the age of 12 months frequent episodes of metabolic decompensations required several hospital admissions. Slight neuromotor retardation occured, but cognitive level was still normal. Because of poor quality of life and poor long-term prognosis under conventional therapy, parents opted for OLT as an alternative strategy.

Patient entered the transplantion list at the age of 30 months and underwent the OLT at the age of 3 years. No complications were observed during the peri-operative period and the child was discharged after 8 days on tacrolimus monotherapy without corticosteroids. Currently, after 2 years post tranplantation, his methylmalonic acid concentrations continue to remain stable under only moderately restricted dietary protein intake (urinary methylmalonic acid concentrations now ranging from 500 to 1000 mmol/molC and serum AMM from 150 to 250 µmol/l). The child achieved full fasting and intercurrent catabolic illness tolerance and gastric drip feeding was no more employed. No more metabolic decompensation has been reported. He showed a marked neuromotor improvement, he started to frequent infant community and he had an important global improvement of quality of life.

In our experience, OLT was an effective treatment and we recommend it to be considered as a terapeutic option in early-onset MMA, so as to improve the prognosis quoad vitam and also to obtain a better quality of life.

Further follow-up is, however, required to study the long-term outcome and to value the effect of OLT on neurological manifestations.

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RESULTS OF LIVER CELL TRANSPLANTATION IN UREA CYCLE DISORDERS

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The urea cycle is the final pathway for the metabolism of waste nitrogen in humans. As many inborn errors of metabolism, urea cycle disorders are based in the liver, but do not damage the liver itself. Indeed, an anomaly of this pathway leads to an accumulation of ammonia, with several deleterious effects to the brain including neurological damage and cognitive deficits. Management by strict diet protein restriction and use of ammonium scavengers are not always sufficient to control the disease and, eventually, the liver remains the target of curative treatment. Orthotopic liver transplantation (OLT) becomes then the ultimate solution to avoid repeated encephalopathic episodes and irreversible brain damage. Rapid transplantation is however limited by the organ shortage and the waiting time is long, during which irreversible brain damage may occur and jeopardize the long-term psychomotor development (1-2).

Liver cell transplantation (LCT) is an emerging therapy that has been shown to give short to medium-term metabolic effects in different diseases (3-4). This technique was proposed as a bridge to OLT in four children with urea cycle disorders, and as an alternative treatment in one because of liver transplant contra-indication (see table).

Four patients had an ornithine transcarbamylase deficiency (OTC), with antenatal diagnosis in two. The first child was transplanted over the first three and a half weeks of life leading to metabolic stabilization from day 20 to 31 (5). On day 31, hyperammonemia reappeared suddenly suggesting that the hepatocytes had been rejected. Despite this setback, the patient was able to receive OLT at 6 months of age to correct the disorder. For the second neonate, blood ammonia was maintained within acceptable limits by combination of hepatocyte transplantation and medical management until 7 months, when the child received an auxiliary liver transplant (6). The third and fourth patients were diagnosed after birth. The third one had undergone several hemodialysis for hyperammonemia resulting in severe limitation of vascular access, eliminating the possibility of liver transplantation7. Metabolic stabilization was obtained in this 5 years old boy after a single infusion of hepatocytes. Unfortunately, he developed an hyperammonemic coma after the

	Disease	Age at LCT	Viable cells	% fresh cells	Immuno-suppression	Follow-up	Outcome
1[5]	OTC	Day 1-23 37-51 113-116	 $4x10^9$ $3.3x10^9$ $1.7x10^9$	 20% 70% 100%	Tacrolimus (5-10ng/ml) + prednisolone	⇑ protein tolerance day20-30 Psychomotor OK	OLT at 6 months No specific complications
2[6]	OTC	Day 1-26	$1.9x10^9$	?	Tacrolimus (5 10ng/ml) + prednisolone	⇓ ammonia levels- ⇑ urea levels Normal diet Psychomotor OK	Auxiliary LT at 7 months No specific complications
3[7]	OTC	5 years	$1x10^9$	100%	?	⇓ ammonia levels Liver biopsies (+) for enzyme activity	Coma hyperNH4 at day 28 (after liver biopsy) Died at day 43
4[8]	OTC	Month 14-18	$2.4x10^9$	0%	Tacrolimus (6-8ng/ml) + prednisolone	⇓ ammonia levels ⇑ urea levels Psychomotor improvement	OLT at 20 months No specific complications
5[9]	ASL	Month 42-47	$3.4x10^9$	50%	Tacrolimus (6-8ng/ml) + prednisolone	⇓ ammonia levels Liver biopsies (+) for donor cells & enzyme activity Psychom catch-up	OLT at 61 months No specific complications

liver biopsy performed 28 days after LCT. He received an additional LCT with cryopreserved cells, but remained comatose despite normalization of the metabolic status. He died of acute bronchopneumonia 43 days after the cell transplantation. The fourth child had experienced numerous attacks of hyperammonemia and was hospital bound. He received LCT between 14 and 18 months, while waiting for liver transplant (8). Ammonia levels decreased, urea synthesis appeared and the psychomotor development improved before OLT.

The fifth child had severe neonatal argininosuccinate lyase deficiency (ASL) (9). Despite optimal medical management, she experienced several peaks of hyperammonemia with secondary psychomotor retardation. She was listed for liver transplantation at the age of 42 months and received hepatocyte transplantations over 5 months. Long-term metabolic stabilization was obtained up to 18 months after the first LCT, when she received a liver transplant. Presence of donor hepatocytes and enzyme activity were demonstrated in her liver. Psychomotor development improved significantly.

In conclusion, liver cell transplantation should be considered as a bridge to OLT in patients with severe urea cycle disorders. Long-term engraftment of donor cells with enzyme activity was demonstrated. Metabolic stabilization was obtained and allowed to decrease the risk of hyperammonemia and neurological sequellae during the waiting time for liver transplant. Additionally, with some further improvements of the technique, LCT could also become an alternative therapy for those diseases. This option would be less radical than liver transplant for patients whom have a normal liver besides the deficient enzyme, and would improve both their prognosis and quality of live.

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PREEMPTIVE LIVER TRANSPLANTATION FOR PRIMARY HYPEROXALURIA TYPE 1

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Hyperoxaluria may be either a secondary or a primary disease. Secondary hyperoxaluria is due to oxalate poisoning, i.e., accidental intoxication with precursors such as ethylene glycol, absorptive hyperoxaluria of bowel disease. Two distinct inherited enzyme defects have been related to type 1 and type 2 primary hyperoxalurias (PH), i.e., alanine : glyoxylate aminotransferase (AGT) and glyoxylate reductase/hydroxypyruvate reductase, respectively ; in addition non-PH1 non-PH2 patients have been reported.

Background

PH1 is one of the most challenging issues for nephrologists. This is an autosomal recessive disorder caused by the functional defect of the hepatic, peroxisomal, pyridoxal phosphate-dependent enzyme AGT leading to oxalate overproduction. The disease occurs because AGT activity is undetectable or because AGT is mistargeted to mitochondria which may explain enzymatic heterogeneity. Since calcium oxalate (CaOx) is insoluble in urine, PH1 usually presents with symptoms referable to the urinary tract. The median age at initial symptoms is 5 to 6 yrs and end-stage renal disease (ESRD) is reached between 25 and 40 yrs of age in half of patients. Along with progressive decline of GFR due to renal parenchymal involvement, continued overproduction of oxalate by the liver along with reduced oxalate excretion by the kidneys leads to a critical saturation point for plasma oxalate (Pox) so that oxalate deposition occurs in many organs, leading to systemic involvement ('oxalosis') and bone is the major compartment of the insoluble oxalate pool. The infantile form often presents as a life-threatening condition because of rapid progression to ESRD due to both early oxalate load and immature GFR : one-half of affected infants experience ESRD at the time of diagnosis and 80 % develop ESRD by the age of 3 yrs.

The aims of conservative therapeutic measures are to increase urinary solubility of CaOx and to decrease oxalate production. Such an aggressive supportive management should be started as soon as the diagnosis of PH1 has been considered that may significantly improve renal survival provided compliance is optimal. Conventional dialysis is unsuitable for patients who have reached ESRD because it cannot overcome the continuous excess production of oxalate.

Organ transplantation

Ideally, any kind of transplantation (Tx) should be a preemptive procedure. Further assessment of oxalate burden needs therefore to be predicted by monitoring sequential GFR, Pox, CaOx saturation and systemic involvement (bone mineral density, bone histology).

Kidney transplantation

Kidney Tx allows significant removal of soluble Pox. However, because the biochemical defect is in the liver, overproduction of oxalate and subsequent deposition in tissues continues unabated. The high rate of urinary oxalate excretion originates from both ongoing oxalate production from the native liver and oxalate deposits in tissues. Due to oxalate accumulation in the graft, isolated kidney Tx is no longer recommended, because recurrence leads to poor graft survival and patient quality of life. On the basis of recent genotype-phenotype studies, such a procedure might be proposed to pyridoxine responsive patients with Gly170Arg mutation but evidence of favorable long term outcome in kidney alone Tx in this subgroup of patients remains to be demonstrated.

Rationale for liver transplantation

Since the liver is the only organ responsible for glyoxylate detoxification by AGT, the excessive production of oxalate will continue as long as the native liver is left in place. Therefore any form of enzyme replacement will succeed only when the deficient host liver has been removed. Liver Tx is a form of gene therapy as well as enzyme replacement therapy as it will supply the missing enzyme in the correct organ, cell and intracellular compartment. The ultimate goal of organ replacement is to change a positive whole-body accretion rate into a negative one by reducing endogenous oxalate synthesis and providing good oxalate clearance via either native or transplanted kidney. However the current reason for enzyme replacement (liver Tx) is only to decrease oxalate, not to cure the disease.

Combined liver-kidney transplantation

In Europe, 6 to 7 combined liver-kidney Tx per year have been reported ; the results are encouraging, as patient survival approximates 80 % at 5 yrs and 69 % at 10 yrs. Comparable results have been reported from the United States Renal Data System, with a 76 % death-censored graft survival at 8 yrs post Tx. In addition, despite the potential risks for the grafted kidney due to oxalate release from the body stores, kidney survival is about 95 % three years post-Tx and the GFR ranges between 40 and 60 mL/min per 1.73m^2 after 5 to 10 yrs.

Isolated liver transplantation

Isolated liver Tx might be the first-choice treatment in selected patients before advanced chronic renal failure has occurred, i.e., at a GFR between 60 and 40 mL/min/1.73m^2. Such a strategy has a strong rationale but raises ethical

Suggestions for transplantation strategies in pyridoxine resistant PH1 patients according to residual GFR, systemic involvement and local facilities (personal opinion)

GFR (mL/min per 1.73m^2)	Estimated Oxalate load	*Proposed Tx strategy*	Comments
60-40	?	Preemptive CAD L-Tx?	Hazardous (hepatectomy, immunosuppression) Limited experience Ethical issues
< 40	+	Preemptive simultaneous (CAD) LK-Tx	HD only if post-Tx ATN/DFG Exposure to immunosuppression
ESRD	++	Simultaneous Tx procedure	Renal risk Pre/post-Tx HD often required
		2-step Tx procedure : 1. L-Tx 2. HD to clear oxalate 3. K-Tx	CAD : needs 2 different donors – loss of immunological benefit LRD : immunological benefit – increased risk for the donor

ATN, acute tubular necrosis
CAD, cadaver
DFG, delayed graft function
ESRD, end-stage renal disease
HD, hemodialysis
K, kidney
L, liver
LRD, living related donor
Tx, transplantation

controversies. Several patients have received an isolated liver transplant without uniformly accepted guidelines, since the course of the disease is unpredictable and a sustained improvement can follow a phase of rapid decrease in GFR.

Post-transplantation reversal of renal and extrarenal involvement

After combined Tx, Pox returns to normal before urine oxalate does, and oxaluria can remain elevated as long as several years. Therefore there is still a risk of recurrent nephrocalcinosis or renal calculi that might jeopardize graft function. Thus, independent of the Tx strategy, the kidney must be protected against the damage that can be induced by heavy oxalate load suddenly released from tissues. Forced fluid intake supported by the use of crystallization inhibitors is the most important approach. Pox, crystalluria and CaOx saturation are helpful tools in renal management after combined liver-kidney Tx. The benefit of daily (pre-) post-Tx hemodialysis is still debated ; it will provide a rapid drop in Pox with an increased risk of CaOx supersaturation in case of reduction in urine volume, and therefore should be limited to patients with significant systemic involvement.

Combined Tx should be planned when the GFR ranges between 20 and 40 mL/min per 1.73m^2 because, at this level, oxalate retention increases rapidly. In ESRD patients, vigorous hemodialysis should be started and urgent liver-kidney Tx should be performed. Even at these late stages, damaged organs do benefit from enzyme replacement, which results in an appreciable improvement in quality of life.

Donors for combined liver-kidney transplantation

The Tx strategy may be based either on immunological bases (i.e., using the same donor for both organs) or on biochemical rationale (i.e., using a two-step procedure according to oxalate body store) (Table). Indeed most publication currently report on the use of cadaver donors but a living related donor may be considered under certain conditions.

Conclusion

Patients with hyperoxaluria should be referred for diagnosis and management to reference centers with interest and experience in the conditions and access to the appropriate biochemical and molecular biological facilities. Indeed major advances in biochemistry, enzymology, genetics and management have been achieved during recent years. The understanding of genotype-phenotype relationship and underlying metabolic defects of PH is in progress. The ongoing analysis of transplant strategies from multicentre database will improve individual enzyme replacement and subsequent patient survival and quality of life.

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LIVER TRANSPLANTATION FOR NEUROLOGIC MANIFESTATIONS OF WILSON'S DISEASE

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Wilson's disease, a copper-overload disease, is an inherited as an autosomal recessive trait. The majority of clinical presentation include hepatic and neurologic manifestations. Hepatic manifestations are represented by end-stage liver cirrhosis or fulminant hepatitis with acute failure. Neurological form is represented by severe motor abnormalities such as ataxia, involuntary movements, hypertonia and loss of speech. Sometime these neurological features lead to bedridden states.

In patients with Wilson disease (WD), liver transplantation (LT) is the therapeutic approach particularly in severe hepatic forms. Moreover LT corrects the metabolic defect and no recurrence after LT has been observed. LT has been increasingly used to treat WD during the last decade. The survival rate after LT is excellent with an overall patient survival rate of 80%.

The indication of liver transplantation for neurological complications, whatever the neurological form remains questionable. The neurological form in its maximal expression leads to severe motor abnormalities such as ataxia, involuntary movements, loss of speech with inability to do simple, casual, daily activities.

Very few reports are available concerning the indication and the outcome of liver transplantation for neurological form of Wilson disease without hepatic manifestations.

In the series of Eghtesad *et al*. a complete neurological improvement occurred in 9 of the 13 survivors. 4 patients did not show complete improvement. Concerning the patients with no improvement, 2 were brothers from the Middle East who presented with advanced, severe neurological manifestations, including dysarthria, tremor, ataxia, and esophageal dysmotility, and in whom D-penicillamine was either ineffective or was associated with unacceptable toxicity. One other patient had a long history of Wilson disease and he underwent LT at 52 years of age.

Our experience of three patients suggests that LT could be benefit in patients with severe neurological forms of Wilson disease. In one patient a dramatic improvement of neurological symptoms was noted three months after liver transplantation. For the two other patients, a slight improvement was noted however at the present time, the neurological condition remains severe.

Of course the existence of predictive factors of a good response of neurological symptoms after liver transplantation must be questionable. First the duration of the neurological symptoms before liver transplantation could represent an important factor. Concerning the patient who recovered, the duration of "fulminant" neurological symptoms was short, (one month), however the duration of neurological abnormalities was as long as in the two other patients. Moreover in the series of Schumacher *et al*. the delay between the first symptom and LT was 11 years.

The kind of neurological symptoms could influence the degree of improvement after liver transplantation. However in the literature and concerning our three cases, the forms are heterogeneous and it would be difficult to obtain a strict conclusion. This question sustains the major problem of Wilson's disease which include different clinical presentation particularly neurological and hepatic forms.

This fact depends probably of genetic factors because of severity and time of presentation of the liver disease or neurological manifestations or sex preponderance are related to the different mutations.

If the results of LT in exclusive neurological forms of the literature and of our short series are quite favorable however the efficacy of LT in neurological forms of WD remain uncertain. Moreover LT is particularly difficult in this kind of patient because of a long delay of hospitalization after LT (median of 3 months concerning our three patients). This fact must include a discussion of a cost/efficacy and the problem of the short supply of liver graft must be taken in account.

At the present time, this program must be evaluated and the different centers of transplantation have to work together to better define the indications of LT and the predictor factors of good response after LT in neurological forms of WD.

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CRIGLER NAJJAR (CN) DISEASE AND LIVER TRANSPLANTATION

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CN disease is caused by an inherited defect of bilirubin conjugation and biliary secretion resulting in an increase of plasma unconjugated bilirubin (UCB) levels. The prognosis is determined by the occurrence of kernicterus caused by toxic levels of free UCB in the brain.
In case of complete absence of bilirubin conjugation this condition start at birth, while residual capacity is associated with milder course and later presentation of the disease.

The backbone for therapy is intense skin irradiation with blue light (460-480 nm) called phototherapy. This energy source cause configurational changes in the UCB molecule resulting in changes of the lipophilic to hydrophilic characteristics associated with increased biliary secretion of UCB. The limitation of this therapy is the intensity, skin characteristics and the compliance of the patient. Increase of free UCB is seen during infectious diseases, accidental hemorrhage, and low albumin binding of UCB by competition with pharmaceuticals. Many of these conditions occur unexpected and are a serious risk for kernicterus. Clinical care needs careful follow up and prophylactic measures. This is very much dependent on the experience of the team. In expert centers it is possible to prevent complications for many years but in single patient care the course is unpredictable and dangerous for the patient.

The ultimate therapy is repair of the genetic defect in the hepatocyte, but this is sofar only achieved in the laboratory setting in animal models. Other possibilities are liver cell transplantation or (partial) liver transplantation.

A world survey showed that the mean time of transplantation was 9 year, but that 26 % of the patients had already neurologic defects at that time. In expert centers it is possible to extend the period to transplantion to young adulthood, but monitoring has to be very careful and finally it is expected that phototherapy alone will be insufficient. Conclusion is that liver transplantation is not a question of if but when.

LIVER OR LIVER CELL TRANSPLANTATION FOR PHENYLKETONURIA

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Phenylketonuria (PKU) is an inherited autosomal recessive disorder (OMIM 261600). The causes of PKU include mutations in the gene encoding phenylalanine hydroxylase (PAH) enzyme or less frequently defect in the metabolism of tetrahydrobiopterin, the cofactor of PAH. PAH is expressed mainly in the liver. It catalyzes the hydroxylation of phenylalanine to tyrosine. Alteration of PAH activity causes an increase in phenylalanine concentrations in blood and tissues, and reduced tyrosine concentrations. Although there is a considerable variation in genotype and phenotype, the great majority of PKU patients are at risk of intellectual and neurological impairements and severe disability. Phenylalanine itself is probably the neurotoxic agent but the exact pathophysiological mechanisms are still unknown. Pathogenesis of PKU can be considered from three viewpoints : a putative deficiency of tyrosine in the brain ; the effect

of hyperphenylalaninemia on transport and distribution of metabolites in the brain and an effect on neurochemical processes, such as defective brain myelination or perturbed protein synthesis. Risk of brain dysfunction due to transient or chronic hyperphenylalaninemia persists throughout life, although risk in adulthood is apparently smaller than in childhood, during brain development.

Several hundred mutations of the PHA gene have been described, inducing various alterations of the protein. This can have an effect at different phenotype levels (enzyme function, phenylalanine homeostasis and brain function). The effect of PAH mutations on enzymatic function can be measured directly by enzyme assay on a liver biopsy or indirectly by expression analysis when the mutation is expressed in a plasmid construct in mammalian or bacterial cell systems. Severity of the clinical phenotype is well correlated with measured or estimated enzymatic activity in the liver. Patients with severe PKU have been shown to have less than 1% of normal activity whereas those with the mild form of the disease had usual more than 5% of normal. Interindividual variation in hepatic enzyme in PKU has been attributed to allelic heterogeneity and possible allelic interaction, usually of negative type. Some asymptomatic heterozygotes had enzyme activity well below expectation (14 to 44 % of the normal instead of the 50 to 100% expected values).

Optimal treatment of PKU requires early onset, continuous drastic restriction of phenylalanine intake. This restriction should be sufficient to keep phenylalaninemia as close as possible to the normal range but sufficient to support protein synthesis. It should be continued throughout childhood and adolescence, and perhaps for life. This markedly reduced protein intake necessitates the use of low protein products and the supplementation with amino acids mixtures selectively free of phenylalanine and enriched in tyrosine. The patients with mild forms of the disease have a greater tolerance for phenylalanine and their regimen are less strict or sometimes unnecessary. Some patients with selected mutation in the PAH gene seem to be further improved by tetrahydrobiopterin supplements. These treatments are expensive ; cost is estimated to be around 1000 euros per month. Tetrahydrobiopterin is very expensive too.

When started in the neonatal period, the treatment modifies the metabolic phenotype and prevents the neuropsychological consequences of hyperphenylalaninemia. Recent studies confirm that dietary treatment achieving in early childhood is compatible with normal executive functions ; the longer and better the control of phenylalanine levels, the better the cognitive, motor and executive function.

Many early-treated PKU patients have however subtle neuropsychological impairements (conceptual tasks, arithmetic skills, motor coordination, ...). Despite these selective deficits, well-treated PKU patients have satisfactory life at the cost of a drastic change of lifestyle on the part of all the family. Whether treatment can be relaxed at adult age is still discussed.

Treatment failures, due to poor compliance for psycho-social reasons, are observed. Quite some patients, mainly with the more severe phenotypes, perform poorly at school age and later in life.

Thus, any technique which allows an increase in PAH activity in the liver up to 5-15% of normal or in another tissue but with comparable efficacy on phenylalanine and tyrosine concentrations in blood might be considered in patients with severe phenotypes and treatment failures, provided it is devoid of side effects.

Considering that increasing PAH activity in the liver to only 5-15 % of normal actually cures the disease, liver cell transplantation should be considered rather than liver transplantation.

Liver cell transplantation is an emerging procedure, consisting of infusing adult hepatocytes in the portal system of the recipient. Best results have so far been obtained in metabolic diseases, such as urea cycle disorders and glycogenosis I. The percentage of engraftment obtained can reach up 10% with de novo expression of deficient activity. Immunosuppressive treatment is necessary in this procedure.

Liver cell transplantation has never been reported to date in PKU patients.

Hamman et al reported recently that transplantation of PAH-positive hepatocytes into PAH-deficient mice (enu2), a model of human PKU, yielded a significant decrease in serum phenylalanine when liver repopulation exceeded approximately 5%. These data confirm that restoration of phenylalanine homeostasis requires PAH activity in only a minority of hepatocytes.

A further step would consist of obtaining the patient's own hepatocytes, transducting them ex vivo with the normal gene in primary culture, and then reimplanting them. A first model of repopulation of rat liver by adult hepatocytes transducted ex vivo with lentiviral vectors was proposed by Oertel et al in 2003.

We conclude that liver cell transplantation could be of interest in the management of patients with severe PKU phenotypes and with treatment failures. It would ameliorate the neurological features and the quality of life of PKU patients. Because risk of brain dysfunction due to hyperphenylalaninemia is higher in early life, this treatment should be proposed as soon as treatment failure is observed.

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