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.

References

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