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Porphyrias: Animal Models and Prospects for Cellular and Gene Therapy

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The rapid progress in the development of molecular technology has resulted in the identification of most of the genes of the heme biosynthesis pathway. Important problems in the pathogenesis and treatment of porphyrias now seem likely to be solved by the possibility of creating animal models and by the transfer of normal genes or cDNAs to target cells. Animal models of porphyrias naturally occur for erythropoietic protoporphyria and congenital erythropoietic porphyria, and different murine models have been or are being created for erythropoietic and hepatic porphyrias. The PBGD knock-out mouse will be useful for the understanding of nervous system dysfunction in acute porphyrias. Murine models of erythropoietic porphyrias are being used for bone-marrow transplantation experiments to study the features of erythropoietic and hepatic abnormalities. Gene transfer experiments have been started *in vitro* to look at the feasibility of somatic gene therapy in erythropoietic porphyrias. In particular, we have documented sufficient gene transfer rate and metabolic correction in different CEP disease cells to indicate that this porphyria is a good candidate for treatment by gene therapy in hematopoietic stem cells. With the rapid advancement of methods that may allow more precise and/or efficient gene targeting, gene therapy will become a new therapeutic option for porphyrias.

KEY WORDS: Porphyrias; animal models; gene therapy.

INTRODUCTION

The Medical Need for Somatic Gene Therapy in Porphyrias

Therapy of porphyrias is generally only partially successful and is often limited to supportive care. This is specially true for the severe forms of porphyrias, e.g. congenital erythropoietic porphyria (CEP), hepatoerythropoietic porphyria (HEP), some cases of erythropoietic protoporphyria (EPP), and homozygous forms of acute hepatic porphyrias (Table 1). Recent developments in the understanding of the molecular basis of porphyrias (see previous chapters) have facilitated novel experimental approaches to therapy. With the availability of the intact genes and cDNAs, animal models can be created and used to study the pathophysiological features of the corresponding human disease. Also, because porphyrias are inherited disorders, they are naturally candidates for somatic gene therapy. The idea underlying gene therapy is that human genetic disease might be treated by transfer of the functional gene into specific cells of the patient (Mulligan, 1993). Although the concept may be very elegant, a number of technical problems and issues have impeded the translation of preclinical studies into effective clinical protocols. First of all, highly efficient gene transfer is required for gene therapy: the mode of introduction of DNA sequences is very important. Two different delivery systems are being developed: viral vectors and nonviral vectors. The second point to be considered is the transduction of

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Table I. Classification of the Major Human Porphyrias and Corresponding Animal Models

Classification	Enzyme deficiency	Animal models
Erythropoietic porphyrias		
Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	Cattle, fox, squirrel, swine, cat (?)
Erythropoietic protoporphyria	Ferrochelatase	Cattle, griseofulvin-treated mouse, mouse[a]
Hepatoerythropoietic porphyria	Uroporphyrinogen decarboxylase	Not known
Hepatic porphyrias		
Acute intermittent porphyria	Porphobilinogen deaminase	Mouse[b]
Hereditary coproporphyria	Coproporphyrinogen oxidase	Not known
Variegate porphyria	Protoporphyrinogen oxidase	Not known
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	Not known

[a] Animal model obtained by chemical mutagenesis.
[b] Animal model obtained by homologous recombination in ES cells.

the appropriate target cells: here also two different approaches are used: the *ex vivo* gene therapy and the *in vivo* administration. Finally, an important issue to be considered is that strict gene regulation is not required for most metabolic disorders (except for diseases such as hemoglobinopathies). This is the case for porphyrias where the disease is the consequence of the accumulation of toxic compounds in different tissues.

In this article, the authors review (a) the different animal models of porphyrias, (b) some general considerations for gene therapy in genetic diseases, and (c) recent progress made in transplantation and gene therapy for erythropoietic porphyrias.

ANIMAL MODELS OF PORPHYRIAS

There are only two genetic porphyrias naturally occurring in animals: erythropoietic protoporphyria (EPP) and congenital erythropoietic porphyria (CEP). Furthermore, two murine models of porphyrias have been created: a model of EPP by chemical mutagenesis and a model of hepatic porphyria (porphobilinogen deaminase or PBGD defect) by homologous recombination in embryonic stem cells (Table I).

EPP in Animals

Bovine Protoporphyria

Bovine EPP is an autosomal recessive disorder (Ruth *et al.*, 1977) while EPP in humans is mostly inherited as an autosomal dominant trait. Affected animals have photosensitivity and marked elevation of free protoporphyrin in red cells and stool. Ferrochelatase activity was shown to be only 10% of the normal value in all tissues investigated from the affected animals (Ruth *et al.*, 1977; Bloomer *et al.*, 1982) and intermediate in heterozygous cattle (Sassa *et al.*, 1981). Immunochemical studies suggest that bovine protoporphyria results from a point mutation in the gene encoding ferrochelatase (Straka *et al.*, 1991). The EPP seems not to be associated with the hepatic features observed in severe human EPP cases (Brenner and Bloomer, 1979) and in the $Fech^{m1Pas}/Fech^{m1Pas}$ mouse (see below). This lack of hepatobiliary disease could be explained, in part, by the enhancement of bile acid secretion which helps maintaining efficient protoporphyrin excretion (Bloomer *et al.*, 1990).

The Protoporphyric Mouse $Fech^{m1Pas}/Fech^{m1Pas}$

An inherited erythropoietic protoporphyria has been obtained in the mouse after a chemical mutagenesis experiment with ethylnitrosourea (Tutois *et al.*, 1991). The $Fech^{m1Pas}/Fech^{m1Pas}$ mouse is characterized by a profound ferrochelatase deficiency: enzyme activity is less than 7% of controls in the different organs tested. The level of the residual enzyme activity is $\sim$ 50% of normal in heterozygotes, confirming the autosomal recessive mode of inheritance of the disease. Molecular analysis performed on the ferrochelatase cDNA demonstrated the presence of a point mutation leading to a methionine-to-lysine substitution at position 98 in the protein, responsible for the enzyme defect (Boulechfar *et al.*, 1993). Photosensitivity is observed in homozygotes with skin lesions on ears and back: this feature is similar to the condition observed in cattle or in human. Hematological parameters have demonstrated that homozygotes

have a normocytic, slightly hypochromic anemia, with a high reticulocyte count. Biochemical parameters show that plasma bilirubin was markedly increased with an elevation of serum alkaline phosphatase and transaminases.

In conclusion, this mutation is responsible for more severe anemia and hepatic dysfunction than the human disease. However, such a severe hepatic phenotype develops in a few EPP patients with terminal hepatic failure (Kappas *et al.*, 1989). In this context, this mutation represents a useful model to study the pathophysiological features of the human disease. Putative risk factors could be identified to predict severe liver injury, and treatments could also be attempted to prevent or to stop the progression of the disease. Finally, the $Fech^{m1Pas}/Fech^{m1Pas}$ mouse represents the first available and convenient model for gene therapy in this porphyria.

CEP in Animals

A rare autosomal recessive form of this disorder has been described in cattle (Levin, 1968). The clinical and biochemical findings are very similar to human CEP with a high excretion of uroporphyrin I and coproporphyrin I in urine. Studies of red cells from affected animals demonstrated a deficiency of uroporphyrinogen III synthase (Levin, 1968). Mean activity of the enzyme from asymptomatic carriers was intermediate to the means of the activities in normal controls and affected animals (Romeo and Levin, 1970a), confirming that the condition is inherited as an autosomal recessive trait. An autosomal dominant disease has been described in swine (Cloue and Stephens, 1944; Jorgensen and With, 1963) and in domestic short-haired cats (Tobias, 1964; Glenn *et al.*, 1968). Another case of feline congenital erythropoietic porphyria was associated with severe anemia and renal disease (Giddens *et al.*, 1975). Measurement of uroporphyrinogen III synthase activity was not performed in these cases. Finally the fox squirrel (*Sciurus niger*) is a species which naturally excretes large amounts of uroporphyrin I in urine and has a low uroporphyrinogen III synthase activity with little or no disability (Levin and Flyger, 1973; Flyger and Levin, 1977).

These different models of CEP are not commonly used laboratory animals. We have started to design a murine animal model of CEP by homologous recombination. As a first step, we built a deleterious version of the gene coding for mouse uroporphyrinogen III synthase (UROIIIS). Starting from a partial genomic clone for mouse UROIIIS (Bensidhoum *et al.*, 1994), which contained the last two exons of the gene, a replacement vector was constructed. The neomycin resistance (Neo^R) gene, from pMC_1 Neo plasmid was used to interrupt one exon of mouse UROIIIS gene, deleting half of the exon and a piece of the next intron. The HS-TK cassette was added at the 3′ end of the construct and should not undergo homologous recombination. The linearized plasmid was introduced by electroporation into embryonic stem (ES) cells which had been provided by Dr. Dierich and Pr Chambon (LGME, Strasbourg, France). After the electroporation, ES cells were grown in selective conditions, in a medium containing G418 and Gancyclovir, to select for homologous recombinants containing the deleterious version of UROIIIS gene. The recombinants were identified by a specific PCR amplification through the deleted exon. The correct structure of the gene was confirmed by Southern blot analysis using as probe (1) a normal genomic fragment which contained the deleted exon and (2) the neomycin cassette which is introduced in the deleterious construct to interrupt the gene. The homologous recombinant ES cells also showed a decrease in UROIIIS enzyme activity. One UROIIIS recombinant ES clone has been injected in mouse blastocystes in order to obtain chimeric animals since ES cells are totipotent embryonic cells that are able to reconstitute every tissue in the embryo. The male chimeras have been crossed with normal mice to obtain heterozygous animals for the deleterious gene. Crossing heterozygotes should give homozygous mice for the deleterious UROIIIS gene, which have to be tested for UROIIIS gene structure and expression at the mRNA and the protein level. These data will be compared with the phenotypic expression of the gene defect by measuring porphyrin accumulation in the different tissues of the animal and analyzing hematologic parameters (anemia, hemolysis) to assess the severity of the disease in the animal model.

The PBGD-Knockout Mouse: a Model of Acute Porphyria

Recently, an animal model of acute porphyria has been created by homologous recombination in ES cells (Lindberg *et al.*, 1994). The authors disrupted the murine porphobilinogen deaminase (PBGD) gene in these cells by inserting the Neo^R gene in different positions. Animals heterozygous for

Table II. Viral and Nonviral Vectors for Gene Transfer into Mammalian Cells

Vectors	Tissue specificity	Transient (T) or stable (S) expression	Application in gene therapy	
			ex-vivo	*In vivo*
Viral				
Retrovirus	Cells in division	S	Yes	?
Adeno-associated virus	Large	S	Yes	?
Adenovirus	Large	T	Yes	Yes
Herpes virus	Nervous system and other tissues	?	Yes	Yes
Nonviral				
Direct injection of DNA	Large	T	No	Yes
Lipofection	Large	T	Yes	Yes
Ligand–DNA conjugates	Targeted tissue	T	No	Yes

disrupted PBGD manifest biochemical features of AIP, e.g., increased excretion of δ-aminolevulinic acid. This model will be very useful for the understanding of nervous system dysfunction in acute porphyrias. However, to our knowledge, no gene therapy project is developed with this model.

SOME CONSIDERATIONS FOR GENE THERAPY IN METABOLIC DISORDERS

When designing approaches to gene therapy for metabolic disorders such as porphyrias, several technical issues must be considered: the development of vectors for gene transfer and the strategies used to deliver genes to specific tissues.

Vectors for Gene Transfer

A number of vectors, both viral and nonviral, have been developed for the transfer of therapeutic genes into target cells. Virus-mediated gene transfer involves the construction of retroviral vectors to obtain recombinant viral particles that will transfer novel genes into cells by the process of infection. Several different viruses have been considered potential vehicles for gene transfer, including retroviruses. These viruses have distinct properties that might be exploited for somatic gene therapy as described in Table II (see review by Mulligan, 1993). The principal issues involved in the development of synthetic viruses for gene therapy consist of eliminating the pathogenic and replicative properties of the parental virus and minimizing the potential for recombination or rearrangement susceptible to forming new viral elements.

DNA-mediated gene transfer (nonviral vectors) involves the introduction of DNA directly into cells. One of the characteristics of this method of transfer is that the DNA is degraded and eliminated by the cell and expression of the gene is only transient (see Table II). The gene is used as a pharmaceutical agent that can be administered repetitively to treat the disease.

Ex vivo Strategy for Gene Transfer

The goal of *ex vivo* gene therapy is to integrate a gene permanently into the target cells while they are in culture to achieve stable expression of the gene after reimplantation of these cells into the patient. This strategy is suitable for hematopoietic stem cells (HSCs) and hepatocytes and is being applied for hemato-immunological and liver diseases.

Adenosine deaminase deficiency is an ideal model for gene therapy targeting HSCs: results from bone marrow transplantation suggest that transfer of the normal ADA gene into HSCs would be sufficient to correct the enzymatic defect. Three gene replacement therapy trials in patients with ADA deficiency have been initiated at the National Institute of Health (Blaese, 1990, 1993), at the San Raffaele Research Institute in Milan, Italy (Bordignon, 1993), and at Introgene BV, the Netherlands (Valerio, 1992, jointly with Hôpital Necker, Paris, France and the Institute for Child Health, London, UK). Most efforts have been focused on HSCs because the transduction and transplantation of these cells would provide a means of ensuring a continuous supply of genetically modified hematopoietic cells during the lifetime of the patient (Fig. 1). However, the transduction of stem cells has proven to be quite difficult, mainly because they are found only in small numbers in bone marrow and peripheral blood and appear to be primarily quiescent. The absence of long-term expression of

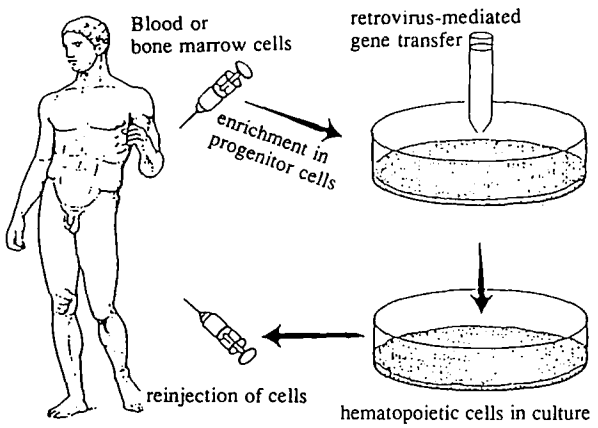

Fig. 1. Strategy of *ex vivo* gene therapy into hematopoietic cells. Bone-marrow or peripheral blood stem cells can be purified by $CD34^+$ cell enrichment on immunoaffinity columns.

the gene in the three patients from the Netherlands, France, and UK was attributed to the absence of conditioning (Hoogerbrugge *et al.*, 1994). By contrast, a long-term survival of transduced cells in two recipients from Milan, Italy was demonstrated by the presence of vector sequences in the DNA extracted from individual T-lymphocyte clones, total peripheral blood mononuclear cells, bone marrow cells, individual bone marrow colonies, and mature granulocytes (Bordignon *et al.*, 1994). In this last case, the relative role of the gene-corrected cells versus the PEG-ADA treatment remains to be determined.

The *ex vivo* strategy for hepatic gene therapy with retroviral vectors is illustrated in Fig. 2. A segment of the liver is removed by partial hepatectomy. Hepatocytes are isolated, grown in primary cell culture, subjected to gene transfer, and then replaced into the body by hepatocellular transplantation. The current genetic model of *ex vivo* hepatic gene transfer is familial hypercholesterolemia, and a clinical trial has been initiated by Wilson (1992). The clinical application of *ex vivo* strategies for hepatic gene therapy is limited because the number of cells that can be successfully engrafted with the recombinant gene is very low. The best available data demonstrate that 20% of the cells removed from the liver can be transformed with retroviral vectors using the *ex vivo* method. Assuming that engraftment represents 5% of the total hepatocytes, ~ 1% of cells in the liver will be transformed. This is sufficient to provide therapeutic benefit for some diseases like familial hypercholesterolemia (Grossman *et al.*, 1994), but therapy for other diseases like hepatic porphyrias may require a much higher transduction efficiency.

In vivo Gene Transfer

Another method for delivering genes is to directly infect or transfect the target tissue with the appropriate vector, thus eliminating the cumbersome steps of explanting, cultivating, and transplanting the target cells. *In vivo* gene delivery using recombinant retrovirus, adenovirus, liposomes, and molecular conjugates have shown promising results in animal models (for review, see Ledley, 1993). But problems with efficiency and stability of recombinant gene expression as well as immune responses to the delivery

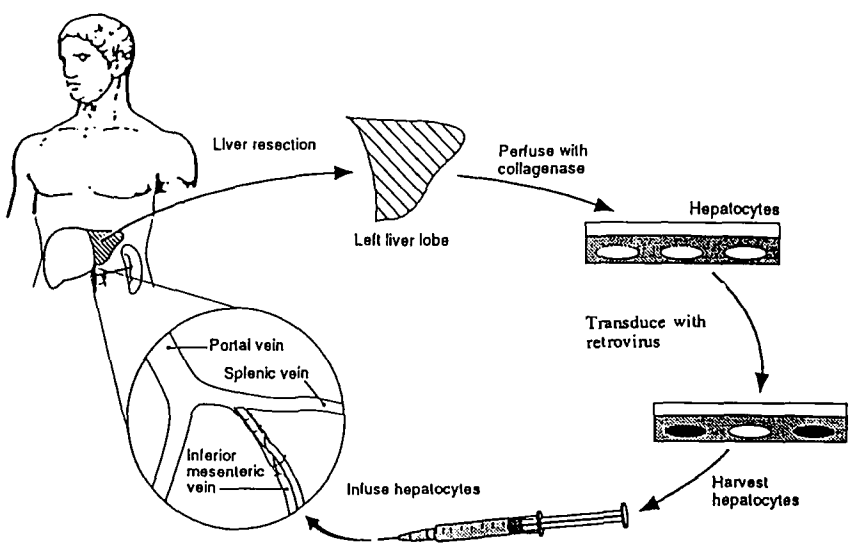

Fig. 2. Strategy of *ex vivo* gene therapy into hepatocytes.

vehicles must be overcome before the potential of *in vivo* approaches can be realized. Recent encouraging data have been published concerning a double-bind trial in nine cystic fibrosis (CF) subjects receiving cationic liposomes complexed with a cDNA encoding the CF transmembrane conductance regulator (CFTR) and six CF subjects receiving only liposomes to the nasal epithelium (Caplen *et al.*, 1995). Transfection efficiency and the duration of expression need to be increased for therapeutic benefit. Another interesting result obtained with this approach is the phenotypic correction of phenylketonuria (PKU) in a genetically deficient mouse by adenovirus-mediated hepatic gene transfer (Fang *et al.*, 1994). However, in this work, repeated administration of recombinant adenoviruses could not duplicate the original effect. It seems clear that new vectors need to be developed for long-term *in vivo* gene therapy of metabolic disorders.

CELLULAR AND GENE THERAPY FOR ERYTHROPOIETIC PORPHYRIAS

Erythropoietic Protoporphyria (EPP)

EPP in humans is characterized by the accumulation of excessive amounts of protoporphyrin in the skin and other tissues, in particular the liver. The disorder is usually transmitted as an autosomal dominant trait with variable penetrance (Kappas *et al.*, 1989) although several cases with a recessive mode of inheritance have been described (Lamoril *et al.*, 1991; Sarkany *et al.*, 1994). A minority of patients develop cholestatis with accumulation of protoporphyrin in hepatobiliary structures and progressive cellular damage when examined by electron microscopy. The etiology of this fatal form of erythropoietic protoporphyria is not understood and is not explained by recessive inheritance because heterozygous cases who developed liver failure have been described (Nakahashi *et al.*, 1993; Schneider-Yin *et al.*, 1994) and one compound heterozygote has been described with normal liver function (Lamoril *et al.*, 1991). Because medical treatment (carotenoids, chenodeoxycholate, hematin) almost invariably fails, most patients with this condition are candidates for liver transplantation (Samuel *et al.*, 1988; Bloomer *et al.*, 1989; Herbert *et al.*, 1991; Mion *et al.*, 1992).

It is generally assumed that erythropoietic tissue is the main source of the excess of protoporphyrin in the patients. However, results of protoporphyrin blood level and fecal excretion before and after transplantation demonstrate that patients appear to be heterogeneous with respect to the contribution of the liver to protoporphyrin production. Experiments of bone-marrow grafting in the $Fech^{m1Pas}/Fech^{m1Pas}$ mouse have been performed (Montagutelli and Deybach, personal communication) in order to determine the respective contribution of the liver and of the erythropoietic tissue to the overproduction of protoporphyrin. This mouse strain represents a useful model to study the features of the hepatic abnormalities and to look at the protoporphyrin production of the liver after cell transplantation or gene therapy of the bone marrow. Mice homozygous for the mutation have received bone marrow cells from normal animals. The accumulation of protoporphyrin in the red blood cells was completely abolished. As a consequence, their level in the plasma was also normalized. In contrast, the level of protoporphyrin in the liver decreased only by a factor of ten, thus remaining one hundred fold over the normal. A similar moderate reduction was also observed in the stool. Since the level of bilirubin was consistently restored to normal, it is likely that the jaundice, observed in the mutant animals, comes from precipitates of protoporphyrin in the biliary ducts, which progressively dissolved following the graft, as the erythron stopped releasing high amounts of protoporphyrin. The protoporphyrin that still remained in the liver was probably that contained in the intracellular crystals that were observed in the histology. The lysis of hepatocytes and canalicular cells persisted at the same level after graft, as indicated by the elevated transaminases and alkaline phosphatase.

This study clearly showed that the replacement of the major part of the mutant bone marrow with normal cells was able to restore the production of normal red blood cells. It also resulted in the abolition of the jaundice. The persistence of the liver disease (accumulation of protoporphyrin and cellular alterations) can be explained by at least three reasons, which are complementary: first, the recipient mutant mice (approximately 3 months old) may have already reached an age when the hepatic lesions are irreversible; second, the liver itself could still be a source of protoporphyrin and contribute to its own alteration; third, because of its enzymatic deficiency, the liver may have been unable to metabolize the protoporphyrin that accumulated firstly from the erythron, and to excrete it in the bile. The last two hypothesis are consistent with the results from the

Table III. Correction of the Enzyme Defect in Cultured Human Erythropoietic Protoporphyria Disease Cells by Retrovirus-Mediated Gene Transfer[a]

Cell type	Ferrochelatase activity (nmol mesozinc/h/mg protein)		Protoporphyrin accumulation Ala (0.5 mM) + Fe (25 μM)
	mean ($n = 3$)	% of normal value	(pmol porphyrin/mg protein/24 h
Normal fibroblasts	10	100	27
Deficient fibroblasts	0.7	7	430
Transduced deficient cells	55	550	124

[a] Deficient fibroblasts transduced with LFSN viral particles carried ~ 0.3 copy per genome.

reverse bone-marrow graft, where normal mice received bone-marrow cells from homozygous mutant donors: the level of protoporphyrin in the erythrocytes reached values close to those observed in homozygous mutant mice. The values for the serum, the liver, and the stool were just slightly above normal. In particular, the increase observed in the liver can be attributed to the red blood cells remaining in the blood vessels of this organ after the sacrifice. The liver did not develop any of the alterations consistently found in the homozygous mutant mice. This could be accounted for by three reasons similar to that mentioned above: first, the age of the recipient mice could be an important factor for the development of the liver disease so that it may be difficult, if not impossible, to induce the jaundice through the accumulation of protoporphyrin after a critical age; second, if the liver plays a key role in its own intoxication, then a normal liver would not be affected, even though the bone marrow produces high amounts of protoporphyrin; third, having a normal ferrochelatase activity, the liver may be able to metabolize the protoporphyrin produced by the erythron, thus avoiding its accumulation. Even though the underlying mechanisms are not yet established, these last results suggest that the long-term prognosis of the transplantation of a normal liver into a protoporphyric patient with liver failure may be good, without reappearance of the liver alterations.

Is There a Place for Gene Therapy in EPP?

Because there are two main target tissues (bone marrow and liver) in this disease, the ideal gene therapy should concern both tissues. However, since the major excess of protoporphyrin production concerns the erythropoietic tissue, gene therapy of HSCs has to be considered in the future, for patients susceptible to develop hepatic complications. When the hepatic disease is present, the hepatic gene therapy seems not to be suitable because of the important damage of the tissues. Liver transplantation remains the treatment of choice.

We have started retrovirus-mediated gene transfer of a human ferrochelatase cDNA into deficient fibroblasts from a compound heterozygous patient. Preliminary results are shown in Table III. Enzymatic activity was increased from 7% (deficient fibroblasts) to 550% (transduced deficient cells) of the normal value. Furthermore, we demonstrated a partial metabolic correction of the disease: porphyrin accumulation in the presence of δ-aminolevulinic acid and iron was reduced three to four times with only 30% of the cells being transduced. Experiments are in progress to use the $Fech^{m1Pas}/Fech^{m1Pas}$ mouse for gene therapy into hematopoietic cells.

Congenital Erythropoietic Porphyria (CEP)

CEP is a rare disease that is inherited as an autosomal recessive trait and is characterized biochemically by a massive porphyrinuria resulting from the accumulation in the bone marrow, peripheral blood, and other organs of large amounts of predominantly type I porphyrins. Diagnosis of CEP patients can be confirmed by the demonstration of a uroporphyrinogen III synthase (UROIIIS) deficiency in erythrocyres and other tissues (Romeo and Levin, 1969; Romeo *et al.*, 1970b; Deybach *et al.*, 1981). The determination of the nucleotide sequence of the cDNA encoding UROIIIS (Tsai *et al.*, 1988) has made possible the study of the molecular lesions responsible for the disease (Deybach *et al.*, 1990; Boulechfar *et al.*, 1992; Warner *et al.*, 1992; Xu *et al.*, 1995; Bensidhoum *et al.*, 1995). The prognosis is poor with death in early adult life and, for some cases, in the neonatal period (Verstraeten *et al.*, 1993; de Verneuil *et al.*, 1995).

Table IV. Correction of the Enzyme Defect in Cultured Human Congenital Erythropoietic Porphyria Disease Cells by Retrovirus-Mediated Gene Transfer[a]

Cell type	UROIIIS activity (nmol urogen III/h/mg protein)		Porphyrin accumulation (ALA : 0.3 mM) pmol porphyrin/10^9 cells/24 h
	mean ± s.d.	% of normal value	mean ± s.d.
Normal fibroblasts	1.65 ± 0.25	100	—
Deficient fibroblasts	< 0.04	< 2	—
Transduced and G-418 selected deficient fibroblasts	31 ± 5.0	1900	—
Normal LB	4.57 ± 0.84	100	4 ± 1.8
Deficient LB	< 0.04	< 1	29 ± 11
Transduced and G-418 selected deficient LB	5.52 ± 0.70	121	1.5 ± 0.9

[a] Deficient fibroblasts transduced with LUSN and selected by G-418 carried ~ 2 copies per genome, whereas transduced and selected deficient lymphoblastoid cells (LB) carried ~ 1 copy per genome.

Available treatments are only symptomatic and unsatisfactory. General treatment includes minimal exposure to the sun, avoidance of trauma to the skin, and careful treatment of infection. Oral carotenoids undoubtedly decrease photosensitivity of patients. Oral treatment with activated charcoal to absorb intraluminal porphyrins in the intestine was effective in reducing the porphyrin concentration and clinical symptoms in some patients. Splenectomy may benefit patients with CEP by reducing the degree of hemolysis and consequently the stimulation of red cell production in the bone marrow.

Cellular and Gene Therapy in CEP

Because the predominant site of metabolic expression of the disease is the erythropoietic system, bone marrow transplantation represents a curative treatment for patients with severe phenotypes. A single patient has been treated by bone-marrow transplantation from an HLA-identical sibling (Kauffman *et al.*, 1991). There was a remarkable amelioration of the porphyria, but the patient died 11 months later from cytomegalovirus infection. Because these fatal complications can occur and because HLA-identical donors are available in only 30% of the cases, somatic gene therapy might be an effective alternative therapeutic strategy. The best approach to somatic gene therapy in this disease involves the use of recombinant retroviral vectors to transduce cells *ex vivo*, followed by autologous transplantation of the genetically modified cells (Fig. 1). We have recently described the construction of two high-titer, amphotropic vectors encoding the normal human UROIIIS cDNA (Moreau-Gaudry *et al.*, 1995a). These vectors are able to correct UROIIIS deficiency in primary human fibroblasts: enzymatic activity was increased from 2% (deficient fibroblasts) to 121–274% of the normal value for the different constructs (Table IV). These first results demonstrated the feasibility of the transfer of this gene in human cells. Because of the predominant site of the disease, transduction and analysis of UROIIIS in hematopoietic cells should be evaluated. Since hematopoietic stem cells are not readily available from CEP patients, we decided to look at the enzymatic and metabolic correction of the porphyric phenotype in a lymphopoietic target cell population (Moreau-Gaudry *et al.*, 1995b). A full restoration of enzymatic activity has been found in transduced and G418-selected deficient cells. We have also demonstrated the metabolic correction of the disease: porphyrin accumulation was reduced to the normal level (Table IV). However, co-culture experiments demonstrated the absence of cross-correction between nontransduced and transduced cells (Moreau-Gaudry *et al.*, 1995b). Except for the case of a selective advantage of corrected cells, these results suggest that gene replacement need to be done on a high percentage of cells for a substantial improvement of the biochemical abnormalities and a subsequent amelioration of the clinical manifestations.

The ideal target cell for gene therapy of CEP should be the myeloid stem cell or the pluripotent

stem cell for long-term therapy. We have also successfully transduced at high efficiency the UROIIIS gene into peripheral blood progenitor cells (PBPCs) *in vivo* exposed to high-dose chemotherapy (Moreau-Gaudry *et al.*, 1995a). The next step of our work is the infection of CD34+ progenitor cells to study viral integration and expression of UROIIIS after long-term bone marrow culture. Also, the future availability of a mouse model of the disease will permit *ex vivo* gene therapy experiments on the entire animal. In conclusion, we have documented sufficient gene transfer rate and metabolic correction in different cells (fibroblasts, EBV-transformed B cell lines, PBPCs) to indicate that CEP is a good candidate for treatment by gene therapy in hematopoietic stem cells.

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