Research Article

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Phase I open label liver-directed gene therapy clinical trial for acute intermittent porphyria

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See Editorial, pages 666–667

Background & Aims: Acute intermittent porphyria (AIP) results from porphobilinogen deaminase (PBGD) haploinsufficiency, which leads to hepatic over-production of the neurotoxic heme precursors porphobilinogen (PBG) and delta-aminolevulinic acid (ALA) and the occurrence of neurovisceral attacks. Severe AIP is a devastating disease that can only be corrected by liver transplantation. Gene therapy represents a promising curative option. The objective of this study was to investigate the safety of a recombinant adeno-associated vector expressing PBGD (rAAV2/5-*PBGD*) administered for the first time in humans for the treatment of AIP.

Methods: In this phase I, open label, dose-escalation, multicenter clinical trial, four cohorts of 2 patients each received a single intravenous injection of the vector ranging from 5×10^{11} to 1.8×10^{13} genome copies/kg. Adverse events and changes in urinary PBG and ALA and in the clinical course of the disease were periodically evaluated prior and after treatment. Viral shedding, immune response against the vector and vector persistence in the liver were investigated.

Results: Treatment was safe in all cases. All patients developed anti-AAV5 neutralizing antibodies but no cellular responses against AAV5 or PBGD were observed. There was a trend towards a reduction of hospitalizations and heme treatments, although ALA and PBG levels remained unchanged. Vector genomes and transgene expression could be detected in the liver one year after therapy.

Conclusions: rAAV2/5-*PBGD* administration is safe but AIP metabolic correction was not achieved at the doses tested in this trial. Notwithstanding, the treatment had a positive impact in clinical outcomes in most patients.

Lay summary: Studies in an acute intermittent porphyria (AIP) animal model have shown that gene delivery of *PBGD* to hepatocytes using an adeno-associated virus vector (rAAV2/5-*PBG*) prevent mice from suffering porphyria acute attacks. In this phase I, open label, dose-escalation, multicenter clinical trial we show that the administration of rAAV2/5-*PBGD* to patients with severe AIP is safe but metabolic correction was not achieved at the doses tested; the treatment, however, had a positive but heterogeneous impact on clinical outcomes among treated patients and 2 out of 8 patients have stopped hematin treatment.

Clinical trial number: The observational phase was registered at Clinicaltrial.gov as NCT 02076763. The interventional phase study was registered at EudraCT as n° 2011-005590-23 and at Clinicaltrial.gov as NCT02082860.

Keywords: Gene therapy; Acute intermittent porphyria; AAV/PBGD; Adeno-associated virus.

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Abbreviations: AIP, acute intermittent porphyria; PBGD, porphobilinogen deaminase; ALAS, 5-aminolevulinic acid synthase; ALA, aminolevulinic acid; PBG, porphobilinogen; AAV, adeno-associated virus; rAAV2/5-*PBGD*, recombinant adeno-associated vector expressing PBGD; PCR, polymerase chain reaction; SF-36, 36-Item short form health survey; BDI-II, Beck depression inventory II; BAI, Beck anxiety inventory.

Introduction

Acute Intermittent Porphyria (AIP) is inherited as an autosomal dominant disorder of the heme biosynthesis pathway [1,2]. AIP is caused by a defect in porphobilinogen deaminase (*PBGD*) gene which spans 10 kb in chromosome 11q23 [3]. More than 370 different mutations of *PBGD* have been described, including missense, nonsense and splicing mutations, as well as deletions and insertions [4,5].

Heme is synthesized in all body cells but mainly in erythroid cells and the liver. In AIP, PBGD enzymatic activity is reduced to about 50% of normal leading to limited capacity to enhance heme synthesis upon increased biosynthetic demands in the liver [1–3]. 5-aminolevulinic acid synthase (ALAS) is the initial and rate-limiting enzyme of heme biosynthesis. In the liver, the ALAS1 enzyme mediates the reaction of glycine with succinyl-CoA to yield aminolevulinic acid (ALA), which is transformed into porphobilinogen (PBG) by aminolevulinic acid dehydratase. PBGD mediates the condensation of PBG to hydroxymethylbilane, which is processed in a stepwise manner to heme, and negatively controls ALAS1 expression. In AIP subjects the heme deficiency taking place under conditions of augmented heme requirements enhances hepatic ALAS1 activity leading to ALA and PBG accumulation [1–3]. These compounds are believed to be responsible for the complex set of neurotoxic symptoms exhibited by AIP patients [6].

AIP is characterized by acute episodes and asymptomatic periods [1,2,6]. AIP patients commonly show high ALA and PBG blood and urinary levels and their concentrations further increase during acute attacks. These episodes are triggered by factors that activate hepatic heme synthesis including exposure to drugs (like barbiturates, sulfonamides), hormonal changes, infections or starvation [1,2,7]. Clinical disease occurs with very low prevalence (1 in 185,000) [8], but epidemiologic figures based on the incidence of acute attacks greatly underestimate the number of individuals with the genetic defect, which in Sweden is as high as 1 in 10,000 [9,10] and 1 in 1675 in France [11], indicating that a large proportion of affected individuals exhibit an asymptomatic form of the disease, in some cases with high ALA and PBG levels in urine [12,13].

Abdominal pain, frequently accompanied by vomiting, diarrhea or constipation, is the most common symptom of acute attacks. Paresthesia and paralysis also occur, and death may result from respiratory paralysis. Other symptoms include seizures, psychotic episodes, tachycardia and hypertension [1,2,7,14]. Current treatment of acute attacks involves intravenous heme (heme arginate–Normosang® in Europe and lyophilized hematin–Panhematin® in USA) infused and/or a high-carbohydrate diet [15].

Most symptomatic patients have only one attack, but approximately 5% women and 3% men with AIP suffer recurrent and frequent attacks, which persist for many years [6]. This form of severe AIP is a devastating condition that significantly affects the quality of life and demands repeated courses of treatment with heme. Although heme represses ALAS, thus blocking heme biosynthesis, it also activates hemeoxygenase-1 (EC:1.14.99.3), which in turn promotes acute attack recurrences and the decline of the therapeutic efficacy [16,17]. Thromboembolic disease and iron overload (a dose of 250 mg of heme arginate contains 22.7 mg of iron) are also side effects associated with repeated courses of this therapy [18]. Even though prophylactic heme appears to be beneficial in patients with recurrent attacks, life-long exposure to drugs for the control of symptoms may cause considerable adverse events that greatly impair quality of life [16,17]. Thus alternative therapies for severe AIP are needed.

Complete biochemical and symptomatic resolution of AIP was observed in all patients after liver transplantation [19]. This observation supports our working hypothesis that therapies aimed at supplementing hepatocytes with the normal version of the *PBGD* gene may correct the disease. Confirming this notion, our studies in murine AIP models showed that liver-directed gene therapy using an AAV vector encoding PBGD under the control of a liver-specific promoter (rAAV2/5-*PBGD*) was able to restore hepatic PBGD activity to normal values and prevented the occurrence of acute attacks [20]. Toxicology studies in mice (unpublished results) and in non-human primates [21] showed that the vector could be administered safely even at high doses. In 2009 the European Medicines Agency granted Orphan Drug Designation to rAAV2/5-*PBGD* for the treatment of AIP. Subsequently we designed and performed a phase I clinical trial in patients with severe AIP to assess feasibility, safety and efficacy of rAAV2/5-*PBGD*. Here, we report the results of this clinical study, which is the first gene therapy trial performed in patients with AIP, and the first to employ an AAV5-based gene therapy product.

Materials and methods

Gene therapy vector

Vector design and production methodologies have already been described [20,21]. The titer of the virus was determined by quantitative PCR and expressed as genome copies/ml (gc/ml) [21].

Trial design and objectives

The study was designed as a phase 1, open label, dose-escalation clinical trial. Since AIP is a rare disease and its clinical presentation very heterogeneous, each patient served as his/her own control. Thus, the study comprised two different phases (Fig. 1); one pre-therapy and the other post-therapy (observational and

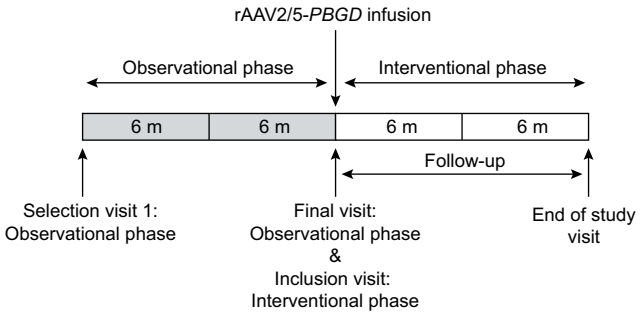

Fig. 1. Study design.