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Pre-clinical efficacy and dosing of an AAV8 vector expressing human methylmalonyl-CoA mutase in a murine model of methylmalonic acidemia (MMA)

Randy J. Chandler^a and Charles P. Venditti^a

^aOrganic Acid Research Section, National Human Genome Research Institute, National Institutes of Health, Bethesda Maryland, USA

Abstract

We demonstrate that human methylmalonyl-CoA mutase (*MUT*), delivered using an AAV serotype 8 vector, rescues the lethal phenotype displayed by mice with MMA and provides long-term phenotypic correction. In addition to defining a lower limit of effective dosing, our studies establish that neither a species barrier to mitochondrial processing nor an apparent immune response to *MUT* limits the murine model as an experimental platform to test the efficacy of human gene therapy vectors for MMA.

Keywords

Methylmalonic Acidemia; MMA; Gene Therapy; adeno-associated virus; AAV

Introduction

Methylmalonic Acidemia (MMA) is an inborn error of propionate metabolism with an estimated incidence of 1 in 50,000 to 100,000 [1]. It is most commonly caused by mutations that impair the activity of methylmalonyl-CoA mutase (*MUT*), the enzyme responsible for the production of succinyl-CoA from L-methylmalonyl-CoA in the mitochondrial matrix [2, 3]. Despite dietary protein restriction and cofactor supplementation, patients with MMA still experience substantial morbidity and mortality [4]. To develop new therapeutics for MMA patients, we have established the pre-clinical efficacy of adeno-associated virus (AAV) mediated gene delivery of the murine *Mut* cDNA in a mouse model of MMA [5, 6]. Our initial experiments focused on viral delivery of the mouse cDNA because substantial amino acid sequence divergence within the mitochondrial localization sequence exists between the human and murine enzymes (Fig. 1A). These amino acid differences could also render the human protein a potential antigen in mice and the murine protein antigenic in humans following gene delivery, reviewed in [7]. In this report, we describe the development and testing of an AAV vector that constitutively expresses the *MUT* cDNA and explore the minimal dosage needed for rescue of the neonatal murine phenotype.

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Materials & Methods

AAV Construction, Production and Purification

The human *MUT* cDNA was cloned into the pENN.AAV2.CBA.CIRBG, a vector that contains a CMV enhancer, chicken beta-actin promoter and a 5' intron, packaged into AAV8 and titered as previously described [8].

Quantitative Real-time PCR

The High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and TaqMan gene expression assays [mouse GAPD (4352932E), murine *Mut* (Mm00485312_m1) and human *MUT* (Hs00165218_m1)] from Applied Biosystems were used for qPCR.

Measurement of Plasma Methylmalonic Acid and *In Vivo* Propionate Oxidation

Plasma methylmalonic acid concentrations were measured as described [9]. *In vivo* 1-¹³C-propionate oxidative capacity was determined by collecting expired ¹³CO₂ [5].

Results and Discussion

AAV8 Delivery of Human *MUT* cDNA Rescues *Mut*^{-/-} Mice

Mut^{-/-} pups (mean weight 1.5 grams, n=8) received a single dose of 1×10⁹ genome copies (GC) (n=10), 1×10¹⁰ GC (n=6) or 2×10¹¹ GC (n=6) AAV8-CBA-MUT by intrahepatic injection at birth. The two highest doses produced a dramatic increase in survival of the *Mut*^{-/-} mice (Fig. 1B) and with a survival rate of greater than 80% for over 9 months. The rescued *Mut*^{-/-} mice appeared and behaved like controls but were slightly smaller in size (not presented), as has been previously documented in studies that used vectors expressing the murine *Mut* cDNA [5, 6]. When the mice received a dose of 1×10⁹ GC of AAV8-CBA-MUT, neonatal rescue was observed but only 50% of the mice survived at one month; this dropped to 10% at 2 months (Fig. 1B). These results suggest that a dose of 1×10⁹ GC of AAV8-CBA-MUT, which is ~7×10¹¹ GC/kg, is approaching the lower limit of AAV required for rescue from neonatal lethality and shows that higher doses are required for a sustained therapeutic effect. Whether this is mediated by reduced transduction or loss of the transgene as a consequence of hepatic growth is under investigation.

Hepatic *MUT* mRNA Expression After Delivery of AAV8-CBA-MUT

Hepatic expression of the *MUT* mRNA of treated *Mut*^{-/-} mice was determined 90 days after treatment. The *Mut*^{-/-} mice that received a dose of 1×10¹⁰ GC (n=3) or 2×10¹¹ GC (n=3) showed a relative *MUT* mRNA expression of 35% and 70%, respectively (Fig. 1C). These results demonstrate a dose-response relationship.

MUT Enzymatic Activity after Delivery of AAV8-CBA-MUT

The concentration of methylmalonic acid, reflective of *MUT* enzyme activity in the treated *Mut*^{-/-} mice, was measured in the plasma following treatment with 1×10¹⁰ (n=6) or 2×10¹¹ GC (n=6) of AAV8-CBA-MUT. The treated *Mut*^{-/-} mice had significantly lower, but not normal, plasma methylmalonic acid concentrations compared to untreated *Mut*^{-/-} mice (n=6,3) at 24 days and 60 days (Fig. 1D) and remained maintained relatively constant between 300-500 μM over time. An independent assay of whole body *MUT* activity, which determines *MUT* activity by measuring the capacity of mice to oxidize 1-¹³C-propionate, was performed on *Mut*^{-/-} mice one year after treatment with 2×10¹¹ GC of AAV8-CBA-MUT. The treated *Mut*^{-/-} mice (n=4) oxidized 41.2±5.5% of the tracer, compared with 12.6±2.2% by untreated *Mut*^{-/-} mice [(n=9) (*P<0.01)] and 65.7±4.6% by untreated *Mut*^{+/+} mice (n=4) (Fig. 1E). Both the ability of treated *Mut*^{-/-} mice to oxidize propionate and

to sustain lower plasma methylmalonic acid levels one year after treatment supports the presence of significant MUT activity.

Conclusion

Neither the elimination of transgene expression nor the impaired mitochondrial importation and processing of the human MUT protein is suggested by our studies. The fact that circulating metabolite concentrations and *in vivo* propionate oxidative capacity in the AAV treated *Mut*^{-/-} mice is almost exactly what we previously observed in studies using AAVs that expressed the murine cDNA provide support for this claim [5, 6].

While the presence of antibodies against MUT was not directly investigated, the prolonged survival after treatment suggests the absence of an immune response. If an immune response to MUT was present, the circulating metabolite concentrations in the AAV treated *Mut*^{-/-} mice likely would have risen over time and/or the treated *Mut*^{-/-} mice would have perished as transgene expression diminished akin to what was observed when *Mut*^{-/-} mice lost transgene expression after adenoviral gene therapy [10] – neither was observed. Alternatively, the mice could be tolerized to the human MUT protein and “ignorant” [11].

Because the MUT antibody was not effective in immunohistochemistry studies, the localization of the enzyme to the mitochondrial matrix could not be verified. However, the facts that patients who harbor mutations that mislocalize MUT are clinically affected, that the enzyme functions in the mitochondrial inner space, and that relatively low levels of hepatic MUT mRNA produced after viral transduction confer long term phenotypic correction provide evidence for mitochondrial localization and full enzymatic function of MUT.

The AAV8-CBA-MUT vector developed for these studies rescued the MMA mice from neonatal lethality, reduced metabolites, restored enzymatic activity, provided metabolic stability, and could be directly translated to human clinical trials.

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Highlights

For the first time an AAV vector compatible for human clinical trials has been validated and a dosing study was performed in a murine model of MMA.

The murine model of MMA has been established as a suitable platform for testing gene therapy vectors suitable for human clinical trials.

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Fig. 1.

(A) Alignment of the human and the murine orthologs of methylmalonyl-CoA mutase. Panel A shows a Clustal W alignment of putative mitochondrial localization signal that is contained first 32 amino acids at the N-terminus of MUT. (B) Survival of *Mut*^{-/-} mice following AAV8-CBA-MUT gene therapy. (C) Hepatic *MUT* mRNA expression in the liver of *Mut*^{-/-} mice 90 days after AAV8-CBA-MUT treatment. Expression of *MUT* was normalized to *Gapdh* mRNA expression and reported as a percentage of endogenous hepatic *Mut* mRNA of *Mut*^{+/+} mice (n=3 in all groups). (D) Decreased plasma methylmalonic acid levels in *Mut*^{-/-} mice treated with either 1x10¹⁰ GC (n=6) or 2x10¹¹ GC (n=6) AAV8-CBA-MUT. Refer to text for details. (E.) Increased propionate oxidation (MUT activity) in AAV8-CBA-MUT treated *Mut*^{-/-} mice one year after treatment (n=4), compared to untreated *Mut*^{-/-} mice (n=9) or *Mut*^{+/+} controls (n=3). Error bars are plus/minus one standard deviation.