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A First Step in Viral Gene Therapy for Muscular Dystrophy

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Introduction

Muscular dystrophies are genetically diverse disorders clinically characterized by progressive weakness of muscles of limbs, cranium, or both and by histologic degeneration and regeneration of muscle without abnormal storage of metabolic products. Advances in molecular genetics have revolutionized our understanding of muscular dystrophies, beginning in 1987 with the identification of mutations leading to dystrophin deficiency in Duchenne's muscular dystrophy [1]. Since then, at least 20 genetically distinct forms of limb-girdle muscular dystrophy (LGMD) have been identified [2]. Four subtypes of autosomal recessive LMGD are the result of mutations in genes encoding sarcoglycans (SGs) α , β , γ , and δ , which form a subcomplex of the dystrophin–glycoprotein complex linked to muscle membranes. The LGMD variants due to SG mutations are LGMD2C (δ -SG), LGME2D (α -SG), LGMD2E (β -SG), and LGMD2F (γ -SG) [3]. Although molecular genetics has been a powerful tool in unraveling the pathogenesis of muscular dystrophies, harnessing the therapeutic potential of molecular biology for these devastating muscle diseases has been technically challenging.

For autosomal recessive diseases with loss of functional protein (e.g., sarcoglycanopathies and dystrophinopathy), gene replacement therapy is a promising approach. Viral delivery of genes was first performed successfully in children with severe combined immunodeficiency by using retroviruses to deliver normal genes to the patients' bone marrow stem cells, which were removed from the children, infected with the virus (transduced), and injected into the bloodstream, where they engrafted and restored normal immune cells [4,5]. Unfortunately, 5 of 20 children treated with retroviral gene therapy developed leukemias because vector insertion into the patients' DNA activated an oncogene, thus exposing one of the potential pitfalls of gene therapy [6]. Other technical obstacles to gene therapy include inefficient gene delivery, immune rejection, and other vector toxicities.

To develop gene therapy for muscular dystrophy, viral and nonviral vectors have been used to deliver genes in experimental cellular and animal models [7]. Adeno-associated viruses (AAVs) are promising viral vectors for gene delivery because they are not known to cause human disease; however, AAVs have a small DNA-carrying capacity and therefore cannot be used to carry large genes such as dystrophin. In contrast, the α -SG gene (*SGCA*) is relatively small and can be packaged into AAVs; therefore, AAV delivery of *SGCA* to patients with LGMD2D is a potentially achievable goal that Mendell and colleagues are pursuing.

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Rating

•Of importance.

Aims

To test the hypothesis that AAV-vector delivery of the *SGCA* gene can produce α -SG protein in muscle of patients with LGMD2D.

Methods

A double-blind randomized controlled trial was performed using an AAV containing the human *SGCA* gene (rAAV1.tMCK.hSGCA) injected unilaterally into extensor digitorum brevis (EDB) muscle of three LGMD2D patients. Saline was injected into the contralateral control side. Patients received a 3-day course of methylprednisolone with gene transfer.

Results

Biopsies of treated EDB muscles obtained 6 weeks (two patients) and 3 months (one patient) revealed four- to fivefold increases in α -SG protein expression and restoration of the SG complex compared with the control side. Muscle fiber diameter was increased in the muscle 3 months after injection. AAV1-neutralizing antibodies were detected in the blood of all three patients. In treated muscles, levels of CD4⁺ and CD8⁺ T cells were not increased, but major histocompatibility complex class I (MHCI) immunostaining was positive whereas scattered foci of mononuclear inflammation showed a marker of programmed cell death. Peripheral blood mononuclear cells (PBMCs) in vitro showed no interferon- γ response to AAV1 capsid peptides or α -SG, except for a mild viral capsid response in one patient's cells. Restimulation with viral capsid protein did not elicit responses in PBMCs. No adverse events were observed clinically.

Discussion

The results of this viral gene therapy study for LGMD2D demonstrated robust α -SG protein expression for up to 3 months after single intramuscular injections into the EDB muscle.

Comments

This thoughtful proof-of-principal study has shown that intramuscular viral-mediated gene therapy can restore α -SG protein in a small foot muscle of LGMD2D patients without adverse clinical events. These findings are encouraging; however, there may be cause for some concern because subclinical signs of immune activation were identified: detection of AAV1-neutralizing antibodies in plasma, scattered inflammatory foci in muscle (although the mononuclear cells showed a marker of cell death), and MHCI expression suggest antigenic presentation at muscle plasma membranes. Consequently, it will be important to monitor immunogenicity of AAV1 vector-mediated gene therapy for muscular dystrophies, particularly as therapy is scaled up to deliver the vectors to larger muscles through multiple injections or systemically through intravascular infusion. Systemic AAV gene delivery already has been applied successfully in mouse models of muscular dystrophy and is being developed in a canine model [7,8]. The dogs revealed a strong T-cell response to AAV2, AAV6, AAV8, and AAV9 [7,8]; however, as noted in an editorial by Clemens et al. [9] accompanying the article by Mendell et al., canine studies of AAV-vector-mediated gene therapy for hemophilia B (factor IX deficiency) failed to predict AAV capsid-induced immune reactions observed in humans, underscoring the need for clinical trials in patients with muscular dystrophy after successful preclinical studies in animals.

In addition to monitoring immune responses, future studies also must assess whether restoration of α -SG protein and SG complexes will lead to functional improvements in muscle strength as a clinically meaningful outcome.

This first-in-human study of vector-mediated gene therapy for LGMD2D is a small and cautious step forward, but a giant conceptual leap toward therapies of muscular dystrophies.

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