Case Report: Treatment of Duchenne Muscular Dystrophy with Intravenous Infusion of Mesenchymal Stem Cells and Exosome Therapies

Darah Mohammed Pourakbar, M.S. Kipp Van Camp, D.O. Cameron D. Knackstedt, D.O.

ABSTRACT

Duchenne muscular dystrophy is a progressive disorder that has an extremely poor long-term prognosis. Few available options offer long-term improvement in symptoms or life expectancy. We treated an 11-year-old male patient with mesenchymal stem cell and exosome therapies. Our goal was to evaluate the safety and effectiveness of such therapy on some of the most common manifestations of Duchenne, including muscle hypertrophy, dexterity, cognition, and balance. At the end of our treatment protocol, the patient showed improvement in these common symptoms.

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder that most commonly affects boys. The majority of patients present between 3 to 5 years of age, most commonly with proximal muscle weakness, gross motor delay, gait abnormalities, and global developmental delay.[1] Progressive symptoms include calf hypertrophy, the inability to jump, dilated cardiomyopathy, cardiac arrhythmias, chronic respiratory insufficiency as a complication of restrictive lung disease, scoliosis, intellectual disabilities, and higher incidences of attention deficit hyperactivity disorders and autism spectrum disorders.[1] Patients with untreated DMD become wheelchair-dependent by early teenage years and die in late teens from respiratory and cardiac complications.[1]

Mutations in the dystrophin genes leads to the development of DMD. The dystrophin gene provides a structural and signaling link between the cytoskeleton of the muscle fiber and the extracellular matrix.[2] Patients with DMD have mutations that cause either loss of the dystrophin protein or the production of a partially functional dystrophin protein.[3] As a complication of this mutation, the muscles of DMD patients undergo chronic cycles of necrosis and regeneration in an attempt to correct the abnormalities brought about by lack of a functional dystrophin protein.[3] The normal muscle regeneration process includes activation of muscle stem cells. Patients with DMD have reduced supply of muscle stem cells secondary to repeated muscle degeneration and regeneration.[3] Lack of muscle stem cells leads to the inability to build new muscle fibers, leading to progressive muscle degeneration.[3]

In addition to progressive muscle degeneration, DMD patients with DMD manifest cognitive deficits. Neurons in the hippocampus, responsible for memory and learning, express dystrophin.[4] Mutations in the dystrophin gene lead to impaired verbal, short-term, and working memory.[4] Although research into the role of dystrophin in the hippocampus is ongoing, the prevailing theory is that either absence of dystrophin or mutated dystrophin leads to malformed and dysfunctional neural synapses that subsequently impair the neural networks responsible for memory creation and processing.[4]

Current treatments for DMD include corticosteroids, cardiac treatment, respiratory support, and physical therapy.[3] There is currently no cure for DMD; these treatments at best slow the progression of the disorder.

Research into the use of gene therapy for treatment of DMD has increased considerably over the past few years. One such treatment is the use of mesenchymal stem/stromal cells (MSCs), mesoderm-derived cells that differentiate into adipogenic, chondrogenic, and osteogenic lineages.[5] In DMD animal models, MSC transplantation showed anti-inflammatory and regenerative activity in damaged muscles.[5] A study by Bier et al. in 2018 showed that MSCs derived from placenta increased the differentiation of human muscle cells from DMD patients and identified exosomes as the mediator of this effect. Through the secretion of exosomes, MSCs are able to promote myoblast fusion and differentiation, leading to decreased creatinine kinase levels, impeded fibrosis, and decreased inflammation.[5] Therefore, when using MSCs to treat DMD, exosomes are added to amplify their effect.

Although there are continuing clinical trials evaluating the safety and efficacy of placenta-derived MSCs, literature searches did not yield a case report or case series that used MSCs in the way our treatment protocol directed. Some small studies have evaluated the use of MSCs in patients for treatment of DMD through different mechanisms. For example, Dai et al. in 2018 conducted a Phase I-II trial on nine male DMD patients who were treated with allogenic Wharton jelly-derived MSCs via intra-arterial and intramuscular administration.[6] The patients in this trial tolerated the treatments well, no serious complications were identified, and some patients had favorable results. However, the main focus of Dai et al. was safety, and their MSCs were derived from Wharton jelly, not placenta.[6] Another study conducted by Klimczak et al. in 2020 evaluated the safety and efficacy of bone marrow-derived MSCs directly transplanted into muscles.[7] Their study also showed favorable results without serious complications. Their delivery method of MSCs was intramuscular, rather than intravenous as our protocol directed, and their MSCs were derived from bone marrow rather than placenta.[7]

Given the promising findings with the use of MSCs in DMD animal models and difference in study design from prior investigations with MSCs, we sought to determine the

safety and efficacy of administering placental and umbilical cord derived MSCs and exosomes via peripheral intravenous infusion in an 11-year-old male patient with DMD.

Case Study

An 11-year-old male patient with DMD was enrolled in an Institutional Review Board (IRB)-approved research protocol. The patient was diagnosed with DMD at age 9 via genetic testing and had previously undergone stem cell-based therapies for the treatment of DMD 9 months prior to enrollment in our treatment protocol. The patient's family had reported that he had developed progressive calf hypertrophy, muscle spasms, increased frequency of falling and tripping, and unsteady gait over the prior 6 months.

Baseline evaluation was performed by an interventional radiologist and included a St. Louis Mental Status Examination (SLUMS), labs, physical exam including musculoskeletal and neurological assessments, pulmonary function testing (PFT), electrocardiogram, MRI imaging of both legs to evaluate gastrocnemius hypertrophy, plain film imaging of the chest and spine, and Duchenne Muscular Dystrophy Musculoskeletal Assessment Form (Table 1).

The patient achieved a normal score of 27 on the SLUMS. Physical exam yielded a normal heart and lung exam. Musculoskeletal exam showed bilateral calf hypertrophy with right calf circumference of 31 centimeters and left calf circumference of 32 centimeters. No additional skeletal asymmetry, atrophy, or hypertrophy were noted throughout the entire muscular skeletal system. Neurological exam showed deep tendon reflexes of 2/4 of the bilateral brachioradial, Achilles, and patellar reflexes. Pulmonary function tests were normal. Laboratory studies included coagulation profile, complete blood count, thyroid studies, and blood chemistries, all of which were within normal limits. Baseline MRI measurement of the calves is shown in Table 2. Electrocardiogram showed no evidence of conduction abnormalities. Chest and spine plain films were within normal limits.

The treatment protocol was administered over a 90-day period. Three consecutive treatments were performed. The first infusion was given on day 0, the second on day 30, and the third on day 60. MSCs and exosomes were mixed with processed platelet-rich plasma (PRP), then infused via peripheral IV over a 60-120 minute interval. Pretreatment with IV diphenhydramine (Benadryl), methylprednisolone, and cephalexin were infused 20-60 minutes before the infusion of MSCs/exosomes.

The DMD Musculoskeletal Assessment Form and the SLUMS mental status test were administered at baseline (first infusion), week 4 (second infusion), week 8 (third infusion), and week 12. MRI imaging of the legs was performed at baseline and at week 12 to evaluate gastrocnemius hypertrophy. The clinician making all these assessments including the MRI evaluation, both at baseline and throughout the treatment protocol, was the same clinician, who was not blinded.

Results

The patient showed promising improvement in several of the evaluated categories. As demonstrated in Table 1, the distance patient was able to walk in 15 seconds increased,

Table 1: Duchenne Muscular Dystrophy (DMD) Musculoskeletal Assessment Form at baseline, week 4, week 8, and week 12.

DMD Assessment Form	Baseline	Week 4	Week 8	Week 12
1. How far can patient walk in 15 seconds?	21-30 ft	>50ft	>50ft	>50ft
2. How long can patient hold his breath?	21-30 sec	31-50 sec	31-40 sec	21-30 sec
3. How fast can patient print his ABC's?	40-60 sec	31-45 sec	31-45 sec	>90 sec
4. How long can patient stand on R leg?	76-90 sec	>90 sec	>90 sec	61-75 sec
5. How long can patient stand on L leg?	>90 sec	>90 sec	>90 sec	>90 sec
6. How long can patient stand on tip toes?	>60 sec	> 60 sec	> 60 sec	> 60 sec
7. How long can patient stand on heels?	0-10 sec	0-10 sec	0-10 sec	0-10 sec
8. How fast can patient say his ABC's?	4-6 sec	4-6 sec	4-6 sec	4-6 sec
9. How many times does patient fall a day?	1-2 times	1-2 times	1-2 times	1-2 times
10. How many times does patient fall a week?	4-6 times	4-6 times	4-6 times	10-12 times
11. How many times does patient trip a day?	1-2 times	1-2 times	1-2 times	1-2 times
12. How many times does patient trip a week?	7-9 times	4-6 times	7-9 times	9-10 times
13. How many claps can patient make in 15 seconds?	58	70	79	74
14. Standing long jump distance R leg	8''	7''	14.5''	16.5''
15. Standing long jump distance L leg	4''	8''	15''	17.5''

Table 2: MRI measurements of calf hypertrophy at baseline and week 12.

MRI Measurements of Calf Hypertrophy	Baseline	Week 12
Right Calf Circumference	31.1cm	30cm
Left Calf Circumference	32cm	31.8cm
Right Calf AP Diameter*	6.5cm	6cm
Left Calf AP Diameter*	6.5cm	6cm
Right Calf AP Diameter Max	7cm	6.5cm
Left Calf AP Diameter Max	8.5cm	8cm
Right Calf Width	8.5cm	8cm
Left Calf Width	9.5cm	9cm

*AP Diameter denotes proximal growth plate of the fibula.

the number of claps patient was able to make in 15 seconds increased, and the distance for standing long jump of both the right and left leg significantly increased. As demonstrated in Table 2, MRI comparisons from baseline to week 12 showed universal improvement in patient's calf hypertrophy including decrease in right and left leg circumference, decrease in right and left leg AP diameter, decrease in right and left leg AP diameter max, and decrease in right and left leg width. Patient maintained a normal SLUMS mental status test at baseline and throughout the entirety of the treatment protocol. The patient tolerated the infusions well with no adverse reactions. Both the patient and his parents reported positive improvement in his overall balance and dexterity from their daily observations throughout the entire protocol. After the protocol was completed, the patient's parents opted to continue treatment with the MSCs and exosome-based therapies.

Discussion

There is currently no cure for DMD, and there are only a handful of treatments currently available that provide any long-term improvement in symptoms and prognosis. As such, there is important continuing research toward finding effective treatment. Stem cell-based therapies have been investigated in animal models as potential treatment options with favorable outcomes. Our 90-day protocol yielded favorable and promising results. The patient tolerated the infusions well with no adverse reactions occurring during the infusions or afterwards.

The patient showed objective improvement in dexterity and balance. There are a few activities, such as the patient's ability to stand on his right leg and the length of time he is able to hold his breath, that seemed to decline as the study progressed. It is unclear whether this decline is significant given the variation in values recorded at the different assessment intervals. Such inconsistencies would be better evaluated in a study that employs this treatment protocol in several patients over a longer time period.

The patient demonstrated objective improvement in calf muscle hypertrophy as well. Although the improvements are small, these authors do consider them significant as there was measurable improvement over a short 12-week treatment period. If such improvement was noted in only 12 weeks, it will be beneficial to evaluate for the extent of improvement over a prolonged treatment course. Our study is limited in that it was conducted on a single patient with all radiographic interpretations made by the same clinician. Future studies should be blinded evaluations in a large number of DMD patients over a prolonged period, with multiple blinded radiologists making assessments for objective radiographic improvement.

An additional consideration in our study is that the patient had previously undergone stem cell-based therapies 9 months prior to enrollment in our treatment protocol. It is unclear whether the patient's improvement was based on our specific study design or on continuation of the previously received stem-cell therapies. However, the patient's family had reported that patient was developing progressive symptoms prior to presentation to our clinic. Such progression of symptoms after stem cell-based therapies were stopped previously points towards the need for prolonged therapy.

One of the most promising methods for treatment of muscular dystrophies lay in stem cell-based therapies.[8] However, their mode of delivery should also be a consideration in their efficacy. Our MSCs were delivered intravenously, and some studies have shown that intravenously injected cells can become trapped in other organs, leading to fewer MSCs reaching their target dystrophic muscle tissue.[8] Clinical trials for cell therapies for DMD have used intramuscular and intra-arterial delivery of stem cells. Arterial delivery in animal models showed more widespread distribution throughout the muscle vasculature.[8] Although our patient did show improvement, intra-arterial injection could potentially yield even better results and is a promising avenue for future studies.

Although stem cell-based therapies are promising, there are drawbacks. Unfortunately, it is a very expensive therapy that is still in the experimental stages of evaluation with

limited long-term data on efficacy.[8] If future studies continue to show prolonged benefit, administration will need to be started at a much younger age to yield maximal effects. [8] Stem cell-based therapies have been shown to be well tolerated and future recommendations on the treatment of muscular dystrophies with such therapies will have to weigh their risks and benefits.

Our results suggest that treatment of DMD with MSCs is effective and safe and can potentially become a DMD treatment that will become the standard of care in years to come. Our study is limited in that it was conducted on a single patient with all assessments made by the same clinician. Future studies should be blinded evaluations of treatment with MSCs in a large number of DMD patients over a prolonged period to assess for safety, efficacy, and long-term benefits with special attention towards comparing the available delivery methods of the MSCs.

Darah Mohammed Pourakbar, M.S., is a medical student. **Kipp van Camp, D.O.,** is a diagnostic and CAQ-trained interventional radiologist practicing at Midwest Regenerative Health and Pain Clinic in Shawnee, Kan. Contact: drkipp1262@gmail.com. **Cameron D. Knackstedt, D.O.,** is a family physician practicing in rural Kansas.

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AAPS Principles of Medical Policy

Medical care is a professional service, not a right. Rights (as to life, liberty, and property) may be defended by force, if necessary. Professional services are subject to economic laws, such as supply and demand, and are not properly procured by force.

Physicians are professionals. Professionals are agents of their patients or clients, not of corporations, government, insurers, or other entities. Professionals act according to their own best judgment, not government "guidelines," which soon become mandates. Physicians' decisions and procedures cannot be dictated by overseers without destroying their professionalism.

Third-party payment introduces conflicts of interest. Physicians are best paid directly by the recipients of their services. The insurer's contract should be only with subscribers, not with physicians. Patients should pay their physician a mutually agreed-upon fee; the insurer should reimburse the subscriber according to the terms of the contract.

Government regulations reduce access to care. Barriers to market entry, and regulations that impose costs and burdens on the provision of care need to be greatly reduced. Examples include insurance mandates, certificate of need, translation requirements, CLIA regulation of physician office laboratories, HIPAA requirements, FDA restrictions on freedom of speech and physicians' judgment, etc.

Honest, publicly accessible pricing and accounting ("transparency") is essential to controlling costs and optimizing access. Government and other third-party payment or price-fixing obscures the true value of a service, which can only be determined by a buyer's willingness to pay. The resulting misallocation of resources creates both waste and unavailability of services.

Confidentiality is essential to good medical care. Trust is the foundation of the patient-physician relationship. Patient confidences should be preserved; information should be released only upon patient informed consent, with rare exceptions determined by law and related to credible immediate threats to the safety or health of others.

Physicians should be treated fairly in licensure, peer review, and other proceedings. Physicians should not fear loss of their livelihood or burdensome legal expenses because of baseless accusations, competitors' malice, hospitals' attempts to silence dissent, or refusal to violate their consciences. They should be accorded both procedural and substantive due process. They do not lose the basic rights enjoyed by Americans simply because of their vocation.

Medical insurance should be voluntary. While everyone has the responsibility to pay for goods and services he uses, insurance is not the only or best way to finance medical care. It greatly increases costs and expenditures. The right to decline to buy a product is the ultimate and necessary protection against low quality, overpriced offerings by monopolistic providers.

Coverage is not care. Health plans deny payment and ration care. Their promises are often broken. The only reliable protection against serious shortages and deterioration of quality is the right of patients to use their own money to buy the care of their choice.