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EDITORIAL COMMENT

Emerging Therapies for Dystrophic Cardiomyopathy*

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Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy that affects approximately 1 of every 5,000 boys born in the United States (1,2). This recessive X-linked genetic disease is a result of mutations in the dystrophin gene. The dystrophin gene is the largest known gene in the human genome as it spans 2.5 Mb, harbors 79 exons, and encodes a 14-kb transcript (2–4). Dystrophin is expressed in the heart (i.e., cardiomyocytes), skeletal muscle, and the brain (2,4). It is a rod-shaped cytoplasmic protein that provides structural stability to the multiprotein dystrophin glycoprotein complex in the plasma membrane and links the cytoskeleton to the extracellular matrix (5). Skeletal muscle lacking functional dystrophin is mechanically weak, and cellular (or sarcomeric) contraction results in membrane damage (6,7). Consequently, the loss of membrane integrity leads to a cascade of increased calcium influx into the cell and eventual cell death.

A majority of the dystrophin mutations are deletions that span 1 or more exons. Clinically, the loss of dystrophin manifests as progressive muscle weakness (5,8). Symptoms are first noted in early childhood, and patients progress to muscle atrophy, contractures, and subsequent loss of ambulation in their early teens. Ultimately, DMD is a lethal and devastating disease. However, with the advent of nocturnal ventilation and spinal stabilization, the life expectancy of young men with DMD has increased to late 20s to early 30s (2,9). Historically, respiratory or pulmonary complications were the leading cause of death in DMD patients, and now dilated cardiomyopathy and sudden cardiac death have emerged as the predominant cause of death in these patients (2,10). Dilated cardiomyopathy is nearly ubiquitous in DMD patients, with approximately 90% of young men over the age of 18 years demonstrating evidence of cardiomyopathy (10). Given the prevalence of cardiomyopathy in this patient population, an enhanced understanding of the pathophysiology of DMD cardiomyopathy is urgently needed.

The most commonly used animal model of DMD is the mdx mouse model (2). This genetic mouse model arose spontaneously and was found to have a point mutation in the dystrophin gene changing glutamine to an early termination codon producing a small, nonfunctional protein (11–13). This mouse model has a robust dystrophic skeletal muscle phenotype involving the diaphragm but otherwise has a relatively mild phenotype that may be due, in part, to the inactivity associated with the animal vivarium. However, the dystrophic skeletal muscle and cardiac phenotypes in the mdx mouse worsen with age (11–13). Although scores of studies using the mdx mouse model have examined the skeletal muscle phenotype, a limited number of studies have examined the cardiac phenotype or examined therapeutic interventions for dystrophic cardiac dysfunction. The high incidence of DMD and dystrophic cardiomyopathy in the general population and the terminal nature of the disease in early adulthood warrant the urgent development of new therapeutic approaches for this disease.

In the paper by Kolwicz et al. (14) in this issue of *JACC: Basic to Translational Science*, the authors used a recombinant adeno-associated viral vector strategy to examine the impact of deoxy-adenosine

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diphosphate elevation or microdystrophin (3.8 kb) in the aged dystrophic heart of the mdx mouse model.

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These studies demonstrated that adeno-associated virus 6 (aAAV6)-mediated delivery of ribonucleotide reductase (RNR, which increases deoxy-adenosine diphosphate and has previously been shown to increase myosin cross bridge binding and cycling resulting in faster contraction and relaxation) or microdystrophin increased cardiac function at baseline and after an increased workload (i.e., pacing stimulation) in aged mdx mice. Furthermore, 5 months post-treatment, mdx mice that received microdystrophin had a marked improvement in cardiac systolic function, whereas mdx mice treated with aAAV6 to specifically overexpress RNR demonstrated improved systolic and diastolic function (14). Importantly, morphological studies verified that aAAV6 delivery of RNR or microdystrophin was localized to the cytoplasmic compartment and the sarcolemma of cardiomyocytes, respectively. In addition, RNR and microdystrophin delivery did not result in worsening cardiac fibrosis or perturbed cytoarchitecture compared with the age-matched mdx control heart (14).

These studies are important for a number of reasons. First, they used a gene therapy strategy that is feasible in the clinical setting. Second, they targeted and compared therapeutic strategies aimed at both the structural and metabolic defects associated with the dystrophic heart. Third, they utilized both the whole animal (mdx mouse) and an in vitro working heart model (i.e., the Langendorff heart preparation) to examine the impact of RNR or microdystrophin on the dystrophic heart function of the mdx mouse. Fourth, the authors examined the impact of RNR or microdystrophin under baseline conditions and after stimulation (i.e., a pacing protocol). Collectively, these studies provide new insights regarding systolic (heart failure with reduced ejection fraction) and diastolic (heart failure with preserved ejection fraction) dysfunction in the dystrophic heart, and they provide a platform for future studies.

Additional studies will be necessary to examine the impact of combined therapies such as the combination of both RNR and microdystrophin (together) on the dystrophic heart. It will also be important to explore the effect of the dystrophic mdx heart (treated with RNR and/or microdystrophin) with other stimuli such as beta-adrenergic stimulation (i.e., chronic isoproterenol stimulation), exercise stimulation (chronic wheel running or a swimming protocol), or other stress-mediated stimuli. Moreover, although the mdx mouse has a relatively mild phenotype, other DMD animal models (i.e., the mdx/utrophin heterozygous mouse model or the dystrophic golden retriever canine model) that reflect the human disease should be used and examined for their response to RNR and/or microdystrophin and their long-term impact on dystrophic cardiomyopathy. Moreover, although the current studies examined the physiological and morphological response to RNR and microdystrophin, future studies should also examine the whole genome response (using bulk RNA sequencing) to treatments directed to structural and metabolic improvements. Finally, studies will need to examine the ability of these therapies to prevent the onset of dystrophic cardiomyopathy as well as the long-term impact of these therapies in the dystrophic background.

In summary, dystrophic cardiomyopathy affects essentially all DMD patients and contributes to their demise. New studies are warranted aimed at increasing our understanding of DMD cardiomyopathy and the development of emerging therapies. The study by Kolwicz et al. (14) is an important step forward in the development of emerging therapies aimed at the structural and metabolic perturbations in the DMD heart.

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