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Of mice and...more mice: The search for an effective treatment for muscular dystrophy using gene therapy

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Gene therapy represents a promising future treatment for Duchenne muscular dystrophy (DMD), the most severe and debilitating form of muscular dystrophy. Mutations in the dystrophin gene are the cause of DMD. Since the dystrophin gene is too large to be packaged in gene therapy vehicles, truncated forms of the gene must be used when administering gene therapy. Previous studies have shown that some of the truncated dystrophin genes capable of ameliorating certain skeletal muscle problems associated with DMD. However, it is not clear whether these truncated genes can improve overall performance. In this experiment, we hypothesize that an optimized mini-dystrophin gene can improve cardiovascular endurance if it is expressed in the heart of the mdx mouse, a mouse model for DMD. A treadmill test was then performed on these transgenic mice and similarly aged controls. Using a Columbus Instruments Treadmill with a shock grid at the base, the mice were trained with increasing intensity for five days, and then tested for overall cardiovascular endurance on the sixth day. Groups of mice were either assigned to an uphill (+7°) or a downhill (-15°) treadmill grade. After training, the mice were placed on a treadmill moving at a medium pace as a warm up for 20 minutes, then the speed was gradually increased until the mouse was exhausted. When a mouse sits on the shock grid for greater than five seconds and fails to reenter the treadmill with gentle prodding, the mouse is defined as exhausted. The transgenic mice consistently ran further, and for a longer period of time than the control mdx mice, which have no dystrophin expression. In the uphill test the transgenic mice ran an average of 603±134.73 meters (n=11), while the mdx mice ran an average of 484.25±89.9 meters (n=4) and the BL10 ran an average of 686.13±77.1 meters (n=8). In the downhill test, the transgenic mice ran an average of 657.78±173.76 meters (n=9), with mdx and BL10 still to be tested. This data suggests that heart specific expression of the minigene may greatly improve cardiovascular health in dystrophic mice. In addition to the mini gene, a micro-dystrophin gene has also been proposed for DMD gene therapy. To further evaluate the therapeutic efficacy of the microgene in skeletal muscle, we performed a second study. In this study, we used a Columbus Instruments Grip Strength Meter to measure the overall force development from limb muscle in the transgenic mice and similarly aged controls. The mice were held by the tip of the tail and allowed to grasp a trapeze bar attached to a force transducer. They were then gently pulled away from the transducer in a horizontal direction to record the peak forelimb grip strength of the mice. Transgenic mdx mice were generated to express the microgene under a skeletal muscle specific promoter. Transgenic mice consistently exhibited higher peak grip strength than the dystrophin lacking mdx mice with a three trial average of 4.42±.45 grams (n=12), while the mdx mice averaged 2.79 ±.43 (n=6) and the BL10 mice averaged 4.44 ±.71 (n=12). The overall forelimb grip strength in the transgenic mice is comparable to that of the wild type BL10 mice. This result suggests that the microgene is sufficient to improve the overall forelimb grip strength of dystrophic mice. Taken together, both mini and micro-dystrophin genes hold great promise for DMD gene therapy.