Combinatorial gene therapy using AAV technology to treat Duchenne muscular dystrophy

T.D. Colgan,[1,2] K.T. Murphy,[1] G.S. Lynch[1] and P. Gregorevic,[2] [1]Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia and [2]Laboratory for Muscle Research & Therapeutics Development, Baker IDI Heart and Diabetes Institute, PO Box 6492, St Kilda Road Central, VIC 8008, Australia.

Duchenne muscular dystrophy (DMD) is a severe and progressive muscle wasting disorder that results in ambulatory reduction of affected children and premature death from cardiac and/or respiratory failure. DMD is caused by a variety of mutations that result in the loss of, or the production of an aberrant dystrophin protein. As DMD is a single gene disorder, gene therapies have been pursued with the intention of restoring dystrophin expression in order to ameliorate the dystrophic pathology. Gregorevic *et al.* (2006) demonstrated the efficacy of a recombinant adeno-associated virus serotype 6 (rAAV6) systemic delivery of microdystrophin. Though the truncated dystrophin gene increased muscle strength and longevity of treated dystrophin$^{-/-}$:utrophin$^{-/-}$ (double knockout: *dko*) mice when compared to their untreated littermates, wild-type levels of strength and lifespan were not obtained. Follistatin binds and inhibits TGF-β ligands myostatin and activin, which are negative regulators of muscle mass. Follistatin has been recently confirmed to mediate increases in muscle growth and strength after systemic delivery (Winbanks *et al.*, 2012). We tested the hypothesis that intramuscular co-delivery of follistatin with microdystrophin would ameliorate the dystrophic pathology to a greater extent than either gene delivered in isolation.

All experiments were conducted in accordance with the code of practise for the care and use of animals for scientific purposes, as stipulated by the NHMRC. To test our hypothesis, cohorts of wild-type C57Bl6, *mdx* and *dko* mice received an intramuscular injection into the right *tibialis anterior* (hindlimb muscle) of either rAAV6:microdystrophin (microdystrophin), rAAV6:FST317 (follistatin) or a combination of both, while contralateral muscles received control vector (rAAV6:MCS). Mice were anaesthetized during the procedure with isoflurane and given a subcutaneous injection of carprofen (100 μL/10 g body mass) for post-recovery pain-relief. Four weeks post-injection, mass gains of 34%, 114% and 168% were recorded for *dko*, *mdx* and C57Bl6 mice respectively, where muscles receiving follistatin were compared to control muscles. Muscles receiving the co-delivery were of a comparable size to muscles only receiving follistatin. To determine why the severity of the dystrophic pathology negatively correlated with the responsiveness of a muscle to follistatin-induced hypertrophy, transgene expression was analysed biochemically. We identified reduced levels of follistatin expression within muscles of treated *dko* mice compared with muscles from *mdx* and C57Bl6. To determine if follistatin expression was reduced due to loss of vector genomes, we performed a time-course analysis of follistatin vector. Although initially similar levels of vector genomes were observed after injection, after two and four weeks these levels were diminished 85-90% in the muscles of dystrophic mice. We hypothesize that the loss of vector genomes is due to increased fibre turnover present in the severe dystrophy model. However, the level of follistatin transgene expression was more abundant in combinatorial treated *dko* muscles than in muscles only treated with follistatin. We must now determine what contributes to the increase in muscle mass and how the increased level of follistatin transgene expression in a combinatorial delivery translates to functional improvements in *dko* mice. These findings are significant as they demonstrate follistatin's capacity to increase muscle mass in a severe dystrophic model and highlight the potential for a combinatorial gene therapy to ameliorate the dystrophic pathology.

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