Thymosin β4 improves the dystrophic pathology in young *mdx* mice

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Duchenne muscular dystrophy (DMD) is the second most commonly occurring genetically inherited disease in humans. It is an X−linked disease that affects approximately one in 3300 live male births. DMD is due to mutations in the gene encoding the protein dystrophin, leading to its absence in skeletal and cardiac muscle, causing loss of the dystrophin-glycoprotein complex and improper mechano-transduction. Dystrophin-deficient muscles are more susceptible to contraction-induced injury, causing necrosis, muscle wasting and premature death. There is currently no effective treatment except palliative therapy with glucocorticoid receptor agonists.

Thymosin β4 (Tβ4) is a 4.9 kDa polypeptide that has been shown to be an important mediator of inflammation, angiogenesis, cell proliferation, migration, and differentiation. A recent study demonstrated limited potential of Tβ4 in treating DMD as chronic treatment of 8-10 weeks of age increased the number of regenerating muscle fibres in muscles of *mdx* mice (Spurney *et al.*, 2010). We tested the hypothesis that Tβ4 treatment commencing at 3 weeks of age would ameliorate the dystrophic pathology to a greater extent than treatments commencing at 8-10 weeks of age.

All experiments were approved by the Animal Ethics Committee of La Trobe University. In order to test this hypothesis, 3 week old *mdx* mice were either treated with Tβ4 (i.p. 6mg/kg) or saline control. During the 6 week treatment, rotarod performance and latency to fall measures were assessed. At the end of the 6 weeks, contractile properties of *tibialis anterior* (TA) muscles *in situ* and diaphragm muscle strips *in vitro* were determined. Using procedures described in detail previously (Gehrig *et al.*, 2012), animals were anaesthetized with sodium pentobarbitone (Nembutal, 60 mg/kg, *i.p.*) prior to assessment of muscle contractile properties and later killed by cardiac excision.

There was no effect of Tβ4 treatment on rotarod or latency to fall performance. Tβ4 had no effect on contractile properties of the TA muscle. However, diaphragm strips from Tβ4 treated *mdx* had an increase in maximum force production as well as an improved recovery rate from fatigue. These improvements in diaphragm function were accompanied by a reduction in fibrosis as measured by histology and gene expression of fibrosis related genes.

These results demonstrate the therapeutic potential of Tβ4 to prevent the progression of the dystrophic pathology rather than reverse established abnormalities. Further pre-clinical studies in additional murine models of DMD may further demonstrate the efficacy of Tβ4 as a treatment option for DMD patients.

Gehrig SM, van der Poel C, Sayer TA, Schertzer JD, Henstridge DC, Church JE, Lamon S, Russell AP, Davies KE, Febbraio MA & Lynch GS. (2012) *Nature*, **484**: 394-398.

Spurney CF, Cha HJ, Sali A, Pandey GS, Pistilli E, Guerron AD, Gordish-Dressman H, Hoffman EP & Nagaraju K. (2010) *PLoS One*, **5**: e8976.