Role of the calcineurin signal transduction pathway in muscle regeneration in dystrophic *mdx* mice

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Whilst sharing the same genotype as in Duchene muscular dystrophy (DMD), *mdx* mice exhibit a more benign dystrophic phenotype. Only the diaphragm muscle of *mdx* mice shows a severe and progressive pathology. Limb muscles of *mdx* mice undergo a bout of severe muscle degeneration at 2 to 4 weeks of age, but a high regenerative capacity ensures almost complete functional and structural recovery (Lynch *et al.*, 2001). The cellular mechanisms responsible for the enhanced regenerative capacity of *mdx* hindlimb muscles are not well understood. Calcineurin, a phosphatase enzyme that regulates transcription by sensing changes in intracellular calcium, has been shown to regulate skeletal muscle regeneration (Sakuma *et al.*, 2003). We have recently shown that inhibiting the calcineurin signal transduction pathway interferes with successful muscle regeneration in young *mdx* mice (Stupka *et al.*, 2002).

When 18 day old *mdx* mice were treated for 16 days with cyclosporine A (CsA; 30 $mg \bullet kg^{-1} \bullet day^{-1}$), an inhibitor of calcineurin, muscle regeneration was severely impaired. EDL and soleus muscle mass was ~25% lower and maximum force producing capacity was 30-35% lower in CsA treated *mdx* mice compared with vehicle treated littermates (Stupka, *et al.*, 2002). In the present study, we performed histological and immunohistochemical analyses to confirm the inhibitory effects of CsA treatment on muscle regeneration in young *mdx* mice.

Muscle sections were stained with haematoxylin and eosin for analysis of general muscle architecture, Van Gieson's stain for collagen deposition, and reacted with antibodies against myogenin (marker of satellite cell differentiation), and macrophages, as markers of regeneration. In CsA treated *mdx* mice, EDL and soleus muscle fibre cross-sectional area was ~25-30% smaller, had fewer centrally nucleated fibres, and more collagen, connective tissue, and mononuclear cell infiltration, than vehicle treated littermates. CsA administration did not affect macrophage infiltration in EDL or soleus muscles from *mdx* mice. Despite having significantly fewer centrally nucleated fibres, EDL and soleus muscles from CsA treated *mdx* mice had two to four times more myogenin positive nuclei than control *mdx* mice. Even though satellite cells from CsA treated *mdx* mice expressed myogenin they did not undergo normal differentiation and myoblast fusion.

Given that the calcineurin signal transduction pathway is essential for successful regeneration of hindlimb muscles in young *mdx* mice, we hypothesise that the pathology of the diaphragm muscle in *mdx* mice may be due to impairment of the calcineurin signal transduction pathway. To test this hypothesis, both upstream and downstream markers of the calcineurin signal transduction pathway in soleus, tibialis anterior, and diaphragm muscles from adult *mdx* and wild type (C57BL/10) mice were examined using a variety of biochemical and immunohistochemical techniques. Preliminary data suggests that there are differences in phosphorylated (inactivated) and dephosphorylated (activated) NFATc1 protein content and calcineurin-A protein content. Understanding the cellular mechanisms responsible for the difference in pathology between *mdx* diaphragm and hind limb muscles may provide insights into the regenerative process of dystrophic muscle and potential novel treatment strategies for DMD.

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