Protein expression and localization of αB-crystallin in muscles of mdx dystrophic mice

N.T. Frankenberg and G.S. Lynch, Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia.

Duchene muscular dystrophy (DMD) is characterized by cycles of degeneration and ineffective regeneration resulting in progressive muscle wasting and weakness. Skeletal muscles are comprised of slow-twitch type I and fast-twitch type II fibres that differ in their metabolic and contractile properties. Studies have shown that degeneration of type II fibres precedes that of type I fibres in DMD (Webster , 1988) and the severity of the disease has been linked to differences in fibre type expression among individual DMD patients (Kleopa , 2006). Protection of the cytoskeleton and cell integrity could be a potential therapeutic target for muscular dystrophy. The small heat shock protein, αB-crystallin, is thought to protect the cytoskeleton and prevent cellular apoposis (Ray , 2011). αB-crystallin can be reversibly phosphorylated and is important in regulating its function. Understanding the cellular characteristics of αB-crystallin in dystrophic muscle could shed light on its therapeutic potential for muscle wasting. We hypothesized that αB-crystallin expression would be upregulated and phosphorylated and that αB-crystallin would be localized to the cytoskeleton in dystrophic muscle.

The present study examined the protein expression of αB-crystallin and phosphorylated αB-crystallin at serine 59 (pαB-crystallin) in fast and slow muscles from control and dystrophic (*mdx*) mice. This study also investigated whether αB-crystallin was interacting with the cytoskeleton. Experiments were approved by the Animal Ethics Committee of The University of Melbourne. Extensor digitorium longus (EDL) and soleus (SOL) muscles were surgically excised from 4 week (wk) and 10 wk old male C57Bl/10 and *mdx* mice that were anaesthetized deeply with Nembutal (120 mg/kg). The mice were killed by cardiac excision. Muscles were snap frozen and Western blotting used to investigate αB-crystallin and pαB-crystallin.

Compared with C57BL/10 mice, there was a ~2.5-fold upregulation of αB-crystallin in the EDL muscle of 4-wk-old *mdx* mice which had decreased by 10 wks of age (n = 4). Interestingly there was no difference in expression in SOL muscles at any age. C57BL10 mice showed no detectable level of phosphorylation in either SOL or EDL muscles. In *mdx* mice there was a ~2-fold and ~14-fold increase of pαB-crystallin in EDL and SOL muscles, respectively at 4 wks but at 10 wk of age no phosphorylation could be detected. In whole muscle homogenates from *mdx* mice the amount of αB-crystallin was ~1-fold and 2.6-fold higher in SOL muscles compared with EDL muscles at 4 and 10 wks respectively. This result was replicated in isolated single muscle fibres where type I fibres expressed ~1.4-fold more αB-crystallin compared with type II fibres at 4 wks of age.

In order to investigate the location of αB-crystallin in muscles of *mdx* mice, mechanically skinned fibres were isolated from the SOL and EDL muscles of 4-wk old C57Bl10 and *mdx* mice. Skinned fibres were exposed to a bathing solution allowing proteins to diffuse out of the fibre. Proteins bound to the cytoskeleton (skinned fibre) and diffusible proteins (bathing solution) were analysed on side-by-side Western blots. In muscle fibres from C57Bl10 mice, 40 ± 13% and 38 ± 12% (mean ± S.D) of αB-crystallin was bound within EDL and SOL muscle fibres respectively, after a 10 min bathing time (n = 4). In fibres from *mdx* mice, 87 ± 8% and 74 ± 5% of αB-crystallin remained within the EDL and SOL fibres respectively, suggesting that the majority of αB-crystallin was bound to cytoskeletal proteins within dystrophic skeletal muscles at 4 wks of age (n = 4).

These findings demonstrate altered protein expression of αB-crystallin in dystrophic skeletal muscles and differences between fast- and slow-twitch muscles. The dramatic increase in αB-crystallin expression, phosphorylation and cytoskeletal localisation in dystrophic muscles identifies its promise as a potential therapeutic target. Investigating these αB-crystallin characteristics will help to elucidate its role in the pathophysiology of muscular dystrophy.

Kleopa KA, Drousiotou A, Mavrikiou E, Ormiston A & Kyriakides T. (2006) *Human Molecular Genetics* **15**, 1623-28

Ray PS, Martin JL, Swanson EA, Otani H, Dillmann WH & Das DK. (2011) *FASEB Journal* **15**, 393-402.

Webster C, Silberstein L, Hays AP & Blau HM. (1988) *Cell* **52**, 503-13.

Supported by the Muscular Dystrophy Association, U.S.A (project grant 255153) and National Health and Medical Research council (project grant APP1009114).