NADPH oxidase: role in muscular dystrophy

N.P. Whitehead and D.G. Allen, Discipline of Physiology, Bosch Institute, University of Sydney, NSW 2006, Australia.

Duchenne muscular dystrophy (DMD) is a degenerative muscle disease caused by the absence of dystrophin, a large (427 kDa) protein connecting the cytoskeleton to the sarcolemma. The dystrophin gene is located on the X chromosome and therefore DMD occurs almost exclusively in males, with an incidence of 1 in 3500 births. In young DMD patients, muscle damage is followed by regeneration but as the disease progresses, regeneration is compromised and muscle fibres are replaced with connective tissue and fatty deposits. This causes profound muscle weakness resulting in loss of mobility by the age of 10-12, and eventually death by about 20, due to respiratory and/or cardiac failure.

Reactive oxygen species (ROS) have been implicated in a wide range of human diseases. Over two decades ago, ROS were considered to be involved in the pathogenesis of DMD, which led to a number of clinical trials using antioxidants. Overall, these trials were disappointing and so the ROS hypothesis lost favour for a number of years. However, recent studies on the *mdx* mouse, an animal model of DMD, have shown that antioxidants can ameliorate dystrophic damage in both skeletal (Hnia *et al*., 2007; Whitehead *et al*., 2008) and cardiac (Williams & Allen, 2007) muscle. These findings suggest that a re-evaluation of the role of ROS in DMD is warranted.

An important follow up question is: what is the source of the increased ROS in dystrophic muscle? Disatnik *et al*., (1998) provided evidence of oxidative stress in *mdx* muscle before the onset of muscle necrosis. This suggests that the primary source of excessive ROS is produced by the dystrophic muscle fibres rather than secondary factors associated with muscle damage, such as inflammatory cells. NADPH oxidase is a ROS-producing, membrane-bound protein complex first discovered in phagocytes. Preliminary evidence from our laboratory suggests that skeletal muscle NADPH oxidase is a primary source of oxidative damage in *mdx* muscle. We have found that the expression of NADPH oxidase proteins, $gp91^{phox}$ (NOX2), $p67^{phox}$ and rac1, is increased approximately 2-fold in pre-necrotic *mdx* muscles compared to wild type. We have also found that NADPH oxidase inhibitors significantly reduce stretch-induced damage in *mdx* muscle fibres. In addition, Jung *et al*., (2008) have recently shown that ROS produced by NADPH oxidase triggers the rise in intracellular Ca^{2+} concentration following hypotonic swelling of *mdx* cardiomyocyes. Finally, Spurney *et al*., (2008) have shown differential gene regulation of NOX isoforms between skeletal and cardiac muscle in *mdx* mice, with NOX2 higher in skeletal muscle and NOX4 greater in cardiac muscle. Since NOX4 is associated with tissue fibrosis, this might explain why *mdx* cardiac muscle undergoes fibrosis while the skeletal muscles are relatively spared. Therefore, this recent experimental evidence suggests that targeting NADPH oxidase could be an effective therapeutic approach for DMD.

Disatnik MH, Dhawan J, Yu Y, Beal MF, Whirl MM, Franco AA & Rando TA (1998). *Journal of Neurological Sciences*, **161**: 77–84.

Hnia K, Hugon G, Rivier F, Masmoudi A, Mercier J & Mornet D (2007). *American Journal of Pathology*, **170**: 633–643.

Jung C, Martins AS, Niggli E & Shirokova N (2008). *Cardiovascular Research*, **77**: 766-773.

Spurney CF, Knoblach S, Pistilli EE, Nagaraju K, Martin GR & Hoffman EP (2008). *Neuromuscular Disorders*, **18**: 371-381.

Whitehead NP, Pham C, Gervasio OL & Allen DG (2008). *Journal of Physiology*, **586**: 2003-2014.

Williams IA & Allen DG (2007). *American Journal of Physiology. Heart and Circulatory Physiolology*, **293**: H1969–H1977.