Contraction-mediated damage in *mdx* dystrophic mouse tibialis anterior muscles is not affected by the membrane sealant poloxamer

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Dystrophin deficiency causes Duchenne muscular dystrophy (DMD), a severe inherited and progressive disease of striated muscle in humans. Dystrophin is a subsarcolemmal protein responsible for linking the cytoskeleton to the extracellular matrix, and it is postulated to play a mechanical role in stabilising the muscle fibre membrane (sarcolemma) during contraction. The muscles of the *mdx* dystrophic mouse, an animal model for DMD, also lack dystrophin, which makes them more susceptible to contraction-induced injury (Dellorusso *et al.*, 2002). The increased susceptibility to stretch-mediated Ca^{2+} overload, leading to cell contracture and death, is prevented by treatment with the membrane sealant poloxamer 188 (P-188; Yasuda *et al.*, 2005). P-188 can incorporate into damaged membranes and effectively 'plug' holes caused by lengthening contractions. We tested the hypothesis that treatment with P-188 would reduce damage and promote membrane integrity in muscles from *mdx* mice following contraction-induced injury.

On the day prior to experimentation, 4-6 month old *mdx* and wild type (C57BL/10 ScSn) mice were injected with Evans blue dye (EBD; 100 mg/kg). Mice were anaesthetised by intraperitoneal injection with pentobarbitone sodium (60 mg/kg), the right external jugular vein exposed, and a bolus dose of P-188 (460 mg/kg body mass; dissolved in 200 µL sterile saline), or vehicle only, infused intravenously. The right tibialis anterior (TA) muscle was surgically exposed and the distal tendon firmly attached to the lever arm of a servomotor/transducer with the knee immobilised by a secure clamp. The right sciatic nerve was also exposed to deliver supramaximal square wave pulses *via* a needle electrode. The TA muscle was immersed in warmed paraffin oil to maintain temperature at 37°C and maximum isometric tetanic tension (P_o) recorded at the muscles optimum length *in situ* (intact nerve and blood supply). The muscle was subjected to two stretches of 40% strain (relative to muscle fibre length; initiated from the plateau of isometric contractions, Consolino & Brooks 2004). The magnitude of damage was assessed 5, 10 and 15 minutes later by the deficit in P_o (force deficit = $(P_{o\ (initial)} - P_{o\ (post\ strain)})/P_{o\ (initial)}$%). The TA muscle was then carefully dissected free and rapidly frozen for later cryosectioning. At the conclusion of experimentation mice were killed by cervical dislocation whilst deeply anaesthetised. Muscle cross sections (8µm) were analysed using a fluorescence microscope for quantification of intracellular infiltration of EBD.

Preliminary findings indicate that force deficit was greater in *mdx* than wild type mice (43 ± 9% vs 25 ± 9%, $P < 0.05$), but was unaffected by P-188 treatment. The proportion of EBD positive fibres was greater in *mdx* than wild type mice (15 ± 7% vs. 2 ± 1%, $P < 0.05$), and was reduced in *mdx* mice treated with P-188 (4 ± 2%, $P < 0.05$), irrespective of injury. The proportion of EBD positive fibres was not affected by the injury protocol in either wild type or *mdx* mice. The results indicate that P-188 does not affect the force deficit following contraction- induced injury but may play a role in maintaining sarcolemmal integrity in muscles from *mdx* mice, which might prevent Ca^{2+} overload and promote cell survival.

Consolino C.M. & Brooks, S.V. (2004) *Journal of Applied Physiology*, **96**, 633-638.

Dellorusso C., Crawford R.W., Chamberlain, J.S., Brooks, S.V. (2002) *Journal of Muscle and Cell Motility* **22**, 467-475. Lee R.C., River, L.P., Pan, F.S., Ji, L., Wollmann, R.L. (1992) *Proceedings of the National Academy of Sciences* **89**, 4524-4528.

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