Myostatin inhibition increases muscle mass in adult *mdx* dystrophic mice but does not enhance regenerative capacity of dystrophic skeletal muscle after injury

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Duchenne muscular dystrophy (DMD) is characterised by continuous cycles of muscle fibre degeneration with less than successful fibre regeneration, leading to progressive muscle wasting, weakness and premature death. There is a profound need to identify therapeutic strategies to improve the dystrophic pathology and enhance quality of life for these patients. Myostatin, a member of the transforming growth factor-β (TGF-β) superfamily, is a negative regulator of muscle mass; high levels of myostatin suppress muscle growth whereas lower levels promote muscle growth (McPherron *et al.*, 1997). Thus, myostatin blockade represents a potential strategy to improve the regenerative capacity and function of dystrophic skeletal muscles. We tested the hypothesis that myostatin inhibition in adult *mdx* mice, a commonly used animal model of DMD, would improve the regenerative capacity and enhance function of dystrophic skeletal muscle.

Male C57Bl/10ScSn *mdx*/J (*mdx*; 12 week old; n=55) mice received weekly subcutaneous injections of either saline or a myostatin antibody (developed by Pfizer Inc., USA; 10 mg/kg) for up to 8 weeks. Five weeks after commencing treatment, mice were anaesthetized deeply (Ketamine, 76 mg/kg, Xylazine, 10 mg/kg, *i.p.*) and the *tibialis anterior* (TA) muscle of the right hindlimb injected with the myotoxin Notexin (~40-50μl; 1 μg/ml in 0.9% saline), to induce complete muscle fibre degeneration. Maximal force production of regenerating muscles was determined at 7, 14, 21 or 28 days post-injury using an *in situ* approach. In addition, for the 28 day post-injury group, the diaphragm was excised from deeply anaesthetized animals (sodium pentobarbital, 60 mg/kg, *i.p.*) and function of isolated diaphragm muscle strips assessed *in vitro* according to methods described in detail previously (Lynch *et al.*, 1997). All mice were killed *via* cardiac excision, while under deep anaesthesia.

The body mass of myostatin antibody treated mice was 13% and 10% greater than that of control mice at 14 and 21 days, respectively ($p < 0.05$). The mass of injured/regenerating TA muscles was greater in the antibody treated group compared with control at 7, 14 and 21 days post-injury ($p < 0.05$), but not at 28 days post-injury. Despite the increase in muscle mass in antibody treated mice, there was no difference in absolute or specific (normalised) force production between groups. There was no difference in specific force production of diaphragm strips between treated and control mice nor did treatment confer protection from contraction-mediated injury, based on muscle force responses during a protocol of repeated lengthening contractions (as described by Schertzer *et al.*, 2007). These data demonstrate that inhibiting myostatin can increase muscle mass in adult *mdx* mice but this may not necessarily translate to an increase in maximal force production.

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