Optimizing methods for ectopic expression of protein in murine skeletal muscle

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Ectopic expression of protein in skeletal muscle has therapeutic potential and is an elegant system for investigating integrative physiology. *In vivo* administration of proteins is often limited by their short half-lives and viral-mediated protein expression methods are complicated by immune responses. Conversely, intramuscular injection of plasmid DNA can induce long-lasting protein expression with a minimal immune response. In this study we assessed a number of different methods using plasmid DNA for ectopic expression of cytokines and growth factors in mouse skeletal muscle. Experiments were carried out on mice that were deeply anaesthetised with sodium pentobarbitone. Methods were tested for maximizing increases in protein levels and minimising muscle damage. Although intramuscular injection of a mammalian expression vector encoding for murine Interleukin-4 (IL-4) alone increased protein levels 275 ± 31% at 7 days post-injection ($p < 0.05$), including a cocktail of Dnase inhibitors (8 mg/mL Aurintricarboxylic acid (ATA), 10 mM $ZnCl_2$, 150 mM $NaPO_4$) increased IL-4 protein levels to a greater extent (564 ± 115%; $p < 0.05$), an effect that was associated with a reduction in muscle-mediated plasmid degradation. However, intramuscular injection of Dnase inhibitors caused significant muscle damage, including muscle fibre degeneration. Subsequently, an electroporation protocol was established that minimised muscle damage and significantly increased protein levels compared to plasmid injection alone. Our studies revealed that an optimal electroporation protocol included injection of 150 Î¼g plasmid DNA (dissolved in a total volume of 40 Î¼L of 50% vol/vol saline) into an adult mouse tibialis anterior muscle followed immediately by 8 electric pulses (50 V/cm, 20 ms each). Increasing the amount of DNA, voltage, or the number of electric pulses increased muscle damage, whereas decreasing these variables reduced plasmid-mediated increases in protein levels. These approaches can now be used to assess the effects of specific cytokines and growth factors in mouse models of muscle pathologies, including muscular dystrophy.

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