Reproducibility and ethical concerns of Notexin as an acute animal injury model

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Muscle wasting is a devastating comorbidity associated with an array of chronic and acute conditions including, but not limited to, injury, diabetes, immobilisation, cancer, ageing, and muscular dystrophies (Jackman & Kandarian, 2004). The use of animal models which accurately and consistently recapitulate the clinical and biochemical signatures of human disease is an essential step in understanding muscle wasting and regeneration.

Notexin is a potent phospholipase A2 toxin derived from Australian Tiger snake venom, commonly used to induce an acute necrotic phenotype (Dixon & Harris, 1996). The current study implemented a relatively low dose intramuscular injection of Notexin solution (40 µl, 10 µg/ml in saline) in the right *tibialis anterior* (TA) and 40 µl saline injected in the left TA of C57Bl/6 mice. Injuries were performed under isoflurane anaesthesia (2-5% flow rate, recovery within 5 minutes) with buprenorphine (0.05mg/kg) injected subcutaneously as analgesic immediately post-injury, and during recovery as prescribed by the supervising veterinarian.

Despite apparent recovery immediately post-injury, all mice exhibited reduced body and behavioural condition by 24-hours post-injury. Constant ongoing surveillance and remedial care was necessary. Mice were culled by CO2 asphyxiation at days 3, 7, and 14 post injury and both TA muscles collected (n=3-4 for each treatment and time point). Tissue architecture and western blot analyses indicated severe and prolonged muscle necrosis.

The adverse events observed in the current study are incongruous with previously reported Notexin injury models (Hardy *et al.*, 2016; Head, Houweling, Chan, Chen, & Hardeman, 2014). These results suggest batch variability in the potency of Notexin, as well as highlighting potential shortcomings in the current reporting of animal injury protocols, with important implications for scientific reproducibility and animal ethics.

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