Effects of the inflammatory agent carrageenan on contractile function in mammalian skeletal muscle

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Inflammation and muscle weakness are two very prominent phenomena that commonly accompany muscle damage and muscle diseases such as Duchenne muscular dystrophy. Whether the inflammatory component of these conditions plays an active role in skeletal muscle weakness is yet to be identified. This question is difficult to address as the effects of inflammation may be confounded by the underlying damage or disease state of the muscle. Lambda carrageenan, an algal-derived polysaccharide, has previously been shown to increase intramuscular levels the pro-inflammatory cytokines IL-1β and IL-6 (Loram *et al.*, 2007). When injected intramuscularly, carrageenan forms a bolus which is localised to the site of injection, thereby providing a model of local muscle inflammation that enables the investigation of the role of inflammatory cytokines in muscle weakness without any confounding influences of preceding muscle damage. This study aimed to determine if the cytokine-mediated inflammatory response directly affected skeletal muscle force production.

All experimental procedures and methods undertaken in this study were approved by the University of Western Australia Animal Ethics Committee. Experiments were conducted on 12 six week old female ARC mice. Each animal received a single 50 µl injection of a lambda-carrageenan solution (2 mg/100 µl saline, lambda carrageenan; Fluka) subcutaneously into the belly of the right tibialis anterior (TA) muscle while anaesthetised (intraperitoneal injection of sodium pentobarbitone, 40 mg/kg body weight). A control injection of 50 µl saline was administered to the contralateral TA muscle. Animals were left for 24 hours to allow carrageenan time to promote an inflammatory response (Loram *et al.*, 2007).

All tissue samples were removed from mice under deep anaesthesia. Mice were anaesthetized *via* an intraperitoneal injection of sodium pentobarbitone (40 mg/kg body weight) such that they were unresponsive to tactile stimuli. Each TA muscle was surgically removed and mounted in an in vitro muscle test system (1200A, Aurora Scientific Inc. Ontario, Canada), bubbled with 95% O_2 and 5% CO_2, whilst bathed in mammalian Ringer solution at 25°C (in mM: 137 NaCl, 24 $NaHCO_3$, 11 glucose, 5 KCl, 2 $CaCl_2$, 1 NaH_2PO_4, 1 $MgSO_4$ and 0.025 d-tubocurarine chloride). The preparation was manually adjusted for optimum muscle length stimulated with supramaximal square-wave pulses by platinum wire electrodes. After removal of the muscles animals were euthanized with an overdose of anaesthetic.

Carrageenan significantly reduced maximum twitch force (~30% reduction in peak twitch tension; $p < 0.05$) and maximal tetanic force (˜20% reduction in peak tetanic tension; $p < 0.05$) when compared to control, saline injected muscles. Furthermore, the force frequency curve was shifted to the right with, carrageenan treated muscles producing significantly less tetanic force compared to saline-treated muscles at 40, 60 and 80 Hz ($p < 0.05$). These results indicate that, in the absence of direct muscle damage, the presence of the inflammatory mediator carrageenan, directly reduces skeletal muscle force production. The effect on the force frequency relationship suggests that the presence of carrageenan may lead to a decrease in net sarcoplasmic reticulum Ca^{2+} release.

Loram LC, Fuller A, Fick FG, Cartmell T, Poole S & Mitchell D. (2007) *The Journal of Pain*, **8:** 127-36.