Anthracycline-induced skeletal muscle weakness: a role for chronic oxidative stress and disrupted calcium homeostasis?

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Doxorubicin (DOX) is an efficacious chemotherapeutic drug, whose use is limited by deleterious side affects. Treatment with DOX can induce severe cardiac cardiotoxicity, such as arrhythmia and heart failure. Current theories concerning the acute mechanisms of doxorubicin-induced cardiotoxicity suggest the drug and its metabolite accumulate in the intracellular Ca^{2+} store (the sarcoplasmic reticulum; SR), targeting SR proteins and altering Ca^{2+} homeostasis, in addition to inducing oxidative stress (Minotti *et al.*, 2004). In both skeletal and cardiac muscle, Ca^{2+} release through the SR Ca^{2+} release channel - the ryanodine receptor (RyR) - triggers muscle contraction. Ca^{2+} is sequestered back into the SR during relaxation though the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA). DOX binds to and alters the function of the cardiac RyR2 isoform and SERCA in the heart (Hanna *et al.*, 2014), contributing to the severe cardiotoxicity. In skeletal muscle, muscular weakness and fatigue are known to accompany DOX treatment. Though the mechanisms of this skeletal muscle debilitation are unknown, it is postulated that DOX treatment induces similar changes in skeletal RyR (RyR1) and SERCA to those underlying the cardiotoxic mechanisms (Gilliam and St Clair, 2011), to disrupt intracellular Ca^{2+} signaling and contribute to this skeletal disorder.

We used an acute DOX treatment model to investigate the effects of DOX treatment on RyR1 and SERCA modification and function; C57BL/6 mice (8-12 week old) were injected with a single dose of DOX (15 mg/kg i.p.), controls received saline i.p. Skeletal muscle weakness was assessed by a wire hang test, prior to mice being euthanized by CO_2 asphyxiation at day 7. SR vesicles (rich in RyR1 and SERCA) were prepared from EDL and *soleus* muscle (Beard and Dulhunty, 2015). SR vesicles were reconstituted into artificial planar lipid bilayers that separate two chambers which are equivalent to the cytoplasmic and SR luminal compartments of the fibre (Hanna *et al.*, 2014), to assess RyR1 function. The impact of DOX treatment on protein expression, protein-protein interactions and stress-induced modification were assessed using SDS-Page, Western blot and thiol probe assay. SERCA function and Ca^{2+} uptake was assessed using an acid-molybdate assay and SR Ca^{2+} release assay, respectively (Hanna *et al.*, 2014).

Mice treated with DOX showed signs of weakness, with a significant decreased latency to fall compared to untreated mice. Treated mice displayed signs of chronic oxidative and phosphorylative stress, with significant increases in RyR1 phosphorylation at Ser^{2844}, with enhanced *S*-nitrosylation and *S*-oxidation in both RyR1 and SERCA from EDL and *soleus*. Functionally, DOX-treated RyR1 from *soleus* and EDL fibres had a higher RyR1 activity under resting conditions, a time when the channel should be closed, which is indicative of RyR1 "leak". SERCA function was compromised in DOX treated skeletal SR vesicles, with a significant decrease in Pi liberation and in rates of Ca^{2+} release in treated mice. Finally, there was a significantly reduced association of regulatory proteins FKBP12.0 and the Ca^{2+} binding protein calsequestin with RyR1 isolated from treated *soleus* and EDL fibres, which was more marked in *soleus* fibres.

The changes in RyR1 thiol modification and channel function are reminiscent of the "leaky" RyR1 phenotype observed in other Ca^{2+} overload myopathies, such as malignant hyperthermia, central core disease, sarcopenia and MDX (Bellinger *et al.*, 2009; Andersson *et al.*, 2011), which result in weakness and/or fatigue. Reduced Ca^{2+} uptake by SERCA would further contribute to cellular Ca^{2+} overload. It is likely that the phosphorylative and oxidative modifications we observed in RyR1 and SERCA from DOX treated mice underlie the RyR1 leak and decreased SERCA re-uptake, as such modifications are well known to contribute to Ca^{2+} overload by compromising RyR1 and SERCA function. Together, these data provide compelling evidence that Ca^{2+} mishandling and chronic stress-induced modification underlie this myopathy, with the individual contributions of these to muscle weakness and fatigue to be determined in the future.

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