Doxorubicin causes lesions in the ETS of skeletal muscle mitochondria which are associated with a loss of contractile function

Background
Doxorubicin is a member of the anthracycline drug class commonly used to treat a range of cancers. Doxorubicin accumulates in skeletal muscle (1), causing myotoxicity, which can persist long beyond the cessation of treatment (2). Patients treated with doxorubicin experience muscle atrophy (2) and a decline in muscle contractile function has been reported prior to muscle atrophy (3) and when normalized to muscle cross-sectional area (CSA) (4). Thus, muscle atrophy alone does not account for the decline in muscle quality.

The concomitant emergence of dysregulated mitochondria following doxorubicin treatment is an emerging area of mitochondrial research, which is dependent upon mitochondrial regulation of cellular energy status and Ca2+ handling. Several mitochondria defects in mitochondrial function may therefore be central to understanding doxorubicin-induced myotoxicity. However, the relationship between doxorubicin and its skeletal muscle mitochondrial quality remains to be fully understood. This is in part due to a lack of diagnostic tools assessing doxorubicin-induced skeletal muscle mitochondriopathy.

The present study was designed to determine the functional impact of acute systemic doxorubicin exposure on skeletal muscle. Measures were conducted 72 hours post-treatment to study the initial phase of dysfunction, prior to extensive muscle wasting, and oxidative damage to proteins and DNA. Fiber-type specific effects were assessed using EDL and soleus muscles due to their divergent fiber-type. In addition, we employed a novel protocol for the assessment of isolated sarcoplasmic reticulum Ca2+ uptake across a physiologically relevant spectra of free energies of ATP hydrolysis (ΔGATP). The study also applied a multiplexed diagnostic assay platform to assess doxorubicin-induced mitochondrial dysfunction under physiological energetic states, which has not previously been explored.

Methods

- Adult C57Bl/6J male mice received either a clinically relevant dose of doxorubicin (DOX) or equal volume of PBS, delivered via intraperitoneal injection, and were euthanized 72 h later.
- Skeletal muscle function was assessed in two different fiber-type divergent muscles (EDL and soleus) using in vitro measures of force, fatigue and contractile kinetics.
- Calcium uptake kinetics were determined fluorometrically using the natural indicator Indo-1, with sarcoplasmic reticulum (SR) isolated from hindlimb muscles.
- High-resolution respirometry measures were conducted in isolated mitochondria under multiple substrate conditions using a modified oxidative phosphorylation assay with various addition of phosphocreatine (PCr) to assess respiratory control (10).
- Simultaneous measures of mitochondrial membrane potential and NAD(P)H/NAD(P) redox potential were conducted under identical substrate and SR-clamp conditions.
- Maximal enzyme activities were determined calorimetrically, and protein content was assessed in intact isolated mitochondria via native gel.

Results

Discussion and Conclusion

Antihypertrophic measures and reductions in EDL and soleus specific force agree with previous studies showing EDL contractile decline in the absence of muscle atrophy measured in G. (5, 6). The underlying cause of increased soleus half-relaxation time is likely due to the noted reductions in SR-dependent Ca2+ uptake following doxorubicin exposure.

Greater caloricoresistive (11) and SERCA sensitivity (12) in the EDL compared to the soleus is likely due to the noted reductions in Ca2+ uptake following doxorubicin exposure. The noted reductions in Ca2+ uptake were found across the spectrum of ΔGATP tested, indicating that mechanisms of doxorubicin impairment are not related to abnormal SERCA sensitivity to cellular ΔGATP.

The substrate-independent nature of the respiratory decline indicates that the limitation is likely not caused by dehydrogenase impairments, nor is it limited to Ca or Cr. Respiratory sensitivity measures the responsiveness of mitochondria to changes in ΔGATP. With the exception of RLC, reduced sensitivity was in line with the overall depression in absolute respiration. As such, the respiratory defect is likely not caused by intrinsic limitations to any one energy phase. Generalized Göksi activity (13) energy and control results in the EDL. Reductions in Cr uptake were found across the spectrum of ΔGATP tested, indicating that mechanisms of doxorubicin impairment are not related to abnormal SERCA sensitivity to cellular ΔGATP.

Reduced membrane potential implies that doxorubicin impairs proton electrochemical gradient across the mitochondrial inner membrane, which is likely an independent mitochondrial defect resides in the ETS. Similar NAD(P)H/NADP⁺ levels were observed for both groups under PCr, GM and PCr/M energized conditions. Plotting ΔGATP against measured NAD(P)H/NADP⁺ revealed no shift in the doxorubicin group, indicating that the activity of the matrix dehydrogenase was not rate limiting in the EDL. Redox changes in Cr uptake were noted in both groups. Quantification of mitochondrial supercomplexes and ATP synthase activity may help to characterize the nature of mitochondrial complex interactions or depletion of ETS components. are the causes of doxorubicin-induced mitochondrialopathy, and doxorubicin associated defects in respiratory function are the subject of ongoing investigation.

Collectively, doxorubicin induces muscle contractile decline that precedes muscle atrophy and is not associated with impaired Ca2+ uptake. Muscle contractile dysfunction is associated with lesions likely spanning complexes IV of the ETC that may provide potential targets for the alleviation of doxorubicin myotoxicity.

References


