Anthracycline-induced dysfunction of cardiac SR Ca\textsuperscript{2+} handling: a potential pathway to anthracycline-induced cardiotoxicity

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Anthracyclines are powerful chemotherapy agents, whose use is limited due to the onset of potentially fatal cardiotoxicity. This cardiotoxicity is complex, manifesting as acute and chronic effects including heart failure and arrhythmia and is thought to have a multifaceted etiology, involving ROS generation, altered Ca\textsuperscript{2+} handling and the accumulation of metabolites. Several proteins important in intracellular Ca\textsuperscript{2+} signaling have been identified as drug binding targets, including the ryanodine receptor Ca\textsuperscript{2+} release channel (RyR2), Ca\textsuperscript{2+} binding protein calsequestrin (CSQ) and the Sarco/Endoplasmic Reticulum Ca-ATPase (SERCA2a). Our previous work found that the anthracycline daunorubicin was able to modulate RyR2 and that these effects were attributable to at least two mechanisms, including ligand binding and thiol oxidation (Hanna et al., 2011).

Hearts were excised from anaesthetized sheep (5% pentobarbitone i.v. followed by oxygen/halothane). RyR2-enriched cardiac SR vesicles were obtained from sheep heart by centrifugation and reconstituted into artificial planar lipid bilayers (Laver et al., 1995). The bilayer was formed across an aperture separating two solutions, equivalent to the cardiomyocyte cytoplasm (cis) and SR lumen (trans). Association of the thiol specific probe Alexa 647 with SR vesicles in the presence and absence of anthracyclines was used to determine whether these drugs modified protein thiols groups. To assess SERCA function, uptake in SR vesicles was monitored spectrophotometrically using the Ca\textsuperscript{2+} indicator dye Antipyrylyazo III. The slope of the Ca\textsuperscript{2+} uptake curve was converted to a rate of uptake using the methods of (Chu et al., 1988).

The functional effect of doxorubicin and its metabolite, doxorubicinol on RyR2 was assessed by adding clinically relevant drug concentrations to single RyR2 channels in lipid bilayers. Both drugs caused biphasic modulation of RyR2 activity where there was an early increase in channel activity followed by a later, inhibitory phase which persisted for the lifetime of the experiment. RyR2 channel activation, but not inhibition, could be reversed by drug washout, typical of a ligand binding effect. This was supported by affinity chromatography experiments showing that doxorubicin and doxorubicinol bind to RyR2. Conversely, the irreversible nature of the inhibitory effect suggested a non-ligand binding effect. Treatment with doxorubicin/doxorubicinol reduced the number of free thiols on RyR2, indicative of a drug-induced thiol-modification such as oxidation. Together, these results support our earlier hypothesis that initial activation of RyR2 by anthracyclines is due to ligand binding, while the inhibitory effect is due to direct thiol-oxidation (Hanna et al., 2011).

In addition, we found that doxorubicinol alters the response of RyR2 to changes in luminal Ca\textsuperscript{2+}. RyR2 activity from untreated channels increased as luminal Ca\textsuperscript{2+} was raised from 0.1 to 1.5 mM. However, following treatment with 2.5 µM doxorubicinol, single RyR2 channels showed no response to changes in luminal [Ca\textsuperscript{2+}]. Further experiments revealed that doxorubicinol-induced thiol oxidation has only a partial contribution to this loss of luminal Ca\textsuperscript{2+} sensing. We have shown that anthracyclines modify RyR2 through at least two different mechanisms and reduce the Ca\textsuperscript{2+} binding capacity of CSQ2. Therefore the direct ligand binding effect of doxorubicinol on either RyR2 or on the major luminal Ca\textsuperscript{2+} sensor, CSQ2 represent potential underlying mechanisms for the loss of RyR2 luminal Ca\textsuperscript{2+} sensitivity. Finally, we found that doxorubicinol inhibits SERCA2a Ca\textsuperscript{2+} uptake into SR vesicles and that this was prevented by pretreatment with DTT. These results provide compelling new evidence that altered Ca\textsuperscript{2+} handling may play a significant role in the onset of anthracycline-mediated arrhythmia and heart failure and reiterate the complexity of anthracycline-induced cardiotoxicity.

