Multiple effects of anthracyclines on cardiac ryanodine receptors

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The anthracyclines doxorubicin and daunorubicin are powerful chemotherapeutics, used effectively in the treatment of various malignancies. The use of anthracyclines is limited however, due to dose-dependent cardiotoxic side effects developing in some patients. These effects present as both acute and chronic impairment of cardiac function and preclude anthracycline use in patients with a pre-existing heart condition. Current theories concerning acute mechanisms of anthracycline-induced cardiotoxicity suggest the drugs and their metabolites, doxorubicinol and daunorubicinol, accumulate in the sarcoplasmic reticulum (SR) of cardiomyocytes where they target SR proteins and cause changes in Ca^{2+} homeostasis. The cardiac ryanodine receptor (RyR2) has been implicated as a target, because the drugs have been shown to dramatically alter SR Ca^{2+} release. However the mechanism of the protein-drug interaction remains unknown and is thought to also involve anthracycline-induced oxidation of critical sulfhydryl groups and generation of free radicals.

Sheep hearts were excised from anaesthetised ewes (5% pentobartitone (IV) followed by oxygen/hatothane). RyR2-enriched cardiac SR vesicles were obtained from sheep heart by centrifugation as described in Laver *et al.* (1995). SR vesicles (containing RyR2 channels) were reconstituted into artificial planar lipid bilayers formed across an aperture separating two solutions, equivalent to the cardiomyocyte cytoplasm (*cis*) and SR lumen (*trans*). The *trans* solution was held at virtual ground and voltage (+40mV or −40mV) was applied to the *cis* solution. Control RyR2 activity was recorded, before drugs were added to the *trans* solution .

Addition of low micromolar concentrations (0.5μM to 10 μM) of anthracyclines to the *trans* (luminal) side of single RyR2 channels caused significant increase in channel activity in a concentration dependent manner. With higher concentrations of each drug, the increase in channel activity was followed by an inhibitory phase where there was a strong reduction in channel activity, which continued for the lifetime of the experiment (up to 30 min). Further investigation of the effects of daunorubicin showed that the activation effect was reversed by washout, but not the inhibitory effect, indicating that the two effects are mediated by separate mechanisms. This was confirmed using the reducing agent dithiothreitol (DTT) and the thiol modifying reagent *N*-ethylmaleimide (NEM), which were able to prevent or reduce the extent of the inhibitory effect but not the activation effect. These data suggest that inhibition of RyR2 activity was caused by oxidation of free thiol groups, likely on the RyR2 itself. Conversely, the reversal of activation following washout and the failure of DTT and NEM to prevent activation, suggests a ligand binding mechanism, either to the RyR2, or an associated regulatory protein.

The results provide novel evidence that anthracycline-induced biphasic modulation of RyR2 channels is mediated by two distinct mechanisms. Firstly, that the activation effect is mediated by a redox-independent mechanism, most likely ligand-binding to either the RyR2 itself or to an associated regulatory protein. Secondly, anthracycline-induced inhibition is caused by drug-induced thiol oxidation Since this anthracycline-induced inhibition was not reversed by DTT we believe that, once formed, the disulfide bond/s become buried in the protein complex, thus inaccessible to DTT. In summary, we provide novel evidence that multiple effects on RyR2 channel activity may contribute to anthracycline-induced disruption of Ca^{2+} homeostasis and subsequent cardiotoxicity. This important new information may help in the eventual design of anthracyclines that do not detrimentally alter Ca^{2+} signalling in the heart.

Laver DR, Roden LD, Ahern GP, Eager KR, Junankar PR and Dulhunty AF (1995) Cytoplasmic Ca2+ inhibits the ryanodine receptor from cardiac muscle. *Journal of Membrane Biology* **147**: 7-22.