Ryanodine receptor dysfunction in anthracycline-induced cardiotoxicity

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Anthracyclines are highly effective chemotherapeutic agents, used to treat various malignancies. However, their use is limited due to the onset of potentially fatal cardiotoxicity which presents with both acute and chronic complications. Current theories surrounding acute cardiotoxicity suggest synergistic effects due to accumulation in cardiomyocytes, where anthracyclines target sarcoplasmic reticulum protein(s), disrupting Ca^{2+} homeostasis. The cardiac ryanodine receptor Ca^{2+} release channel (RyR2), is thought to be partly modulated by anthracycline-induced oxidation of critical sulfhydryl groups. In the present experiments, luminal (*trans*) addition of daunorubicin to RyR2 in lipid bilayers elicited a biphasic response, initially activating then inhibiting the channel. The initial daunorubicin-induced activation, but not the inhibition, was reversible with drug washout (N=8). The reducing agent dithiothreitol (DTT) prevented RyR2 inhibition but not activation, consistent with an oxidation-induced inhibition process. Interestingly, DTT added to the cytoplasmic (*cis*) side of the chamber (but not the *trans* chamber) protected RyR2 from daunorubicin-induced inhibition. This implies daunorubicin crosses the bilayer and oxidizes thiols in the cytoplasmic domain of RyR2, causing inhibition (N=8). DTT added after daunorubicin failed to reverse this anthracyclines-induced inhibition (N=10), suggesting that upon oxidation, the modified thiols become buried within the RyR2 and inaccessible to DTT. The failure of DTT to prevent activation and washout-induced reversibility of activation suggest a ligand-binding mechanism, to either the RyR2 or an associated regulatory protein. Together these results implicate a high affinity ligand-binding action of anthracyclines on the RyR2 complex and that sulfhydryl oxidation is important in anthracycline-induced RyR2 inhibition. The results demonstrate that multiple mechanisms lead to anthracycline-induced disruption of RyR-dependent Ca^{2+} homeostasis and contribution to subsequent cardiotoxicity.