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Real-time optical mapping of acute mitochondrial dysfunction with chemotherapy agent doxorubicin in Langendorff-perfused rat hearts

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Rationale

Cardiotoxicity as a result of the agents used to treat neoplastic disorders has become increasingly clinically relevant. Mitochondria are recognised as a target and potential mediator of this toxicity. Here, we investigate the effect of doxorubicin (DOX) on mitochondria in *ex vivo* and *in vivo* models.

Methods and Results

A semi-quantitative imaging technique of optical mitochondrial membrane potential ($\Delta\Psi m$) mapping in Langendorff-perfused rat hearts using the $\Delta\Psi m$-sensitive dye TMRM was optimized. To validate that TMRM fluorescence intensity reduces with decreases in $\Delta\Psi m$, hearts were perfused with the mitochondrial uncoupling agent FCCP 10μM for 20 min as a positive control; TMRM fluorescence decreased by 98% after 60 min (0.019±0.011 FCCP vs. 0.998±0.008 control: n=8; $P<0.001$). Study hearts were perfused with Krebs-Henseleit solution containing DOX 1μM or 10μM for 60 min. TMRM fluorescence decreased by 12.5% in DOX 1μM-treated hearts after 60 min (0.875±0.032 DOX 1μM vs. 1.02±0.008 control: n=8; $P<0.001$) and by 24% in DOX 10μM-treated hearts (0.763±0.02 DOX 10μM vs. 1.02±0.008 control: n=8; $P<0.001$). Male Sprague-Dawley rats were administered eight weekly 1.25mg/kg intravenous DOX injections. Heart failure phenotype was established by week 11 with a significant reduction in left ventricular ejection fraction (49.6±3.81% DOX n=7 vs. 80.5±3.12% control n=4; $P<0.01$) and marked mitochondrial morphological changes assessed by transmission electron microscopy.

Conclusions

TMRM fluorescence decreased in an acute concentration-dependent manner in DOX-treated hearts suggesting ΔΨm dissipation and mitochondrial dysfunction detectable at the intact organ level. This acute effect indicates that mitochondrial dysfunction may be an early indicator of cardiotoxicity and a potential therapeutic target. A chronic DOX-induced cardiomyopathy model has been established in our institution with the aim of assessing mitochondrially-targeted cardioprotective strategies.