Title: Hypoxic preconditioning protects adult cardiomyocytes from Doxorubicin-induced injury.

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Introduction

Anthracyclines cause chronic irreversible cardiac failure, ascribed variously to generation of reactive oxygen species (ROS), alterations of mitochondrial transmembrane potential ($\Delta\Psi_m$) and opening of the mitochondrial permeability transition pore (mPTP). Recent data suggest cardiac damage begins early and may in fact be a continuum. Cardioprotective modalities against acute injury may therefore provide prolonged benefit.

Hypoxic preconditioning (HP), i.e.: subjecting an organ to brief periods of hypoxia followed by reoxygenation before a prolonged lethal hypoxic insult, protects cardiomyocytes against cell death by activating the pro-survival Reperfusion Injury Salvage Kinases (RISK) pathway. We examined the hypothesis that HP can protect cardiomyocytes against anthracycline-toxicity (Doxorubicin; Dox) in a model using primary rat cardiomyocytes.

Methods

Cardiomyocytes were subjected to HP *in vitro* (Dox^{HP}) or to control protocol before incubating with Dox for 18h and cell death measured by Propidium Iodide. Components of the RISK pathway such as PI3 kinase and ERK1/2 were inhibited in preconditioned cells using LY294002 ($Dox^{LY\text{-}HP}$), and PD98059 ($Dox^{PD\text{-}HP}$) respectively. ROS-involvement in Dox-cardiotoxicity was probed by co-incubating with the ROS scavenger N-acetyl cysteine (NAC) (Dox^{NAC}). Direct ROS-imaging was also carried out using the dye DCF. $\Delta\Psi_m$ and induction of the mPTP was estimated using tetramethyl rhodamine methyl ester (TMRM).

Results and Discussion

HP protected against Dox-toxicity (35.4 $\pm$ 1.7% Dox vs 14.7 $\pm$ 1.5% Dox^{HP}. $p<0.05$, n=5). This protection was abrogated by LY294002 but not PD98059 (16.9 $\pm$ 1.5% Dox^{HP} vs 38.5 $\pm$ 3.3% $Dox^{LY\text{-}HP}$ vs 21.1 $\pm$ 1% $Dox^{PD\text{-}H}$. $p<0.05$ n=5). No significant difference in ROS or $\Delta\Psi_m$ were seen at any time-point, and NAC failed to rescue Dox-induced death (Dox 40 $\pm$ 3.7% vs Dox^{NAC} 41.4 $\pm$ 2.8%. $p>0.05$,n=3). Furthermore, mPTP sensitivity was unchanged by Dox-treatment (Dox 842 $\pm$ 103 sec vs Control 972 $\pm$ 74 sec. $p>0.05$, n=7). These suggest that ROS and mPTP are not involved in protection.

Conclusions and future directions

HP appears to protect cardiomyocyte from Dox-toxicity in an *in vitro* model through the PI3K/Akt component of the RISK pathway. Protection appears to be independent of ROS or mPTP. The mechanism(s) involved merit further investigation.