SIMVASTATIN AND DOXORUBICIN CO-TREATMENT REDUCES DOX-INDUCED OXIDATIVE STRESS AND APOPTOSIS

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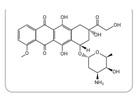
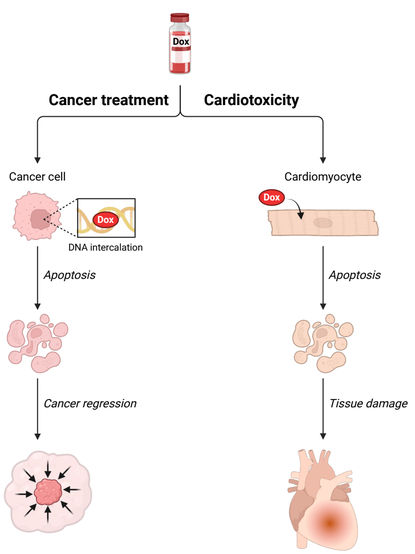
INTRODUCTION

Doxorubicin
Target & Off-Target Effects



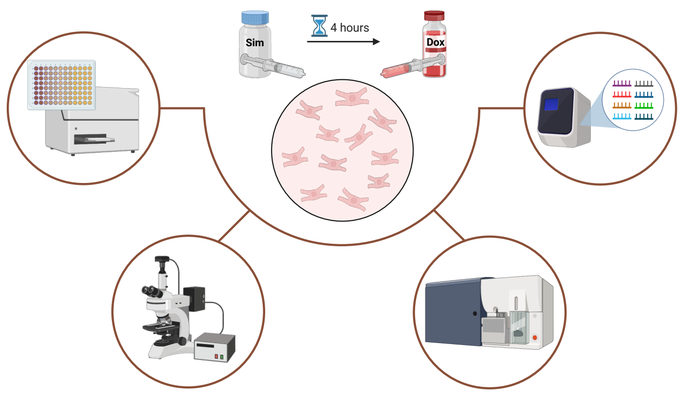


Doxorubicin (DOX)-induced cardiotoxicity is a major cause of long-term morbidity and mortality among cancer survivors. Recent studies described DOX-induced cardiotoxicity as a continuous phenomenon, starting with myocardial cell damage, followed by a progressive functional decline, which gradually leads to overt heart failure. Evidence recognized a central role of oxidative stress and inflammation, which appear to be mutually related. The discovery of therapeutic strategies able to act in the early phase are needed. Drug repurposing may represent a promising approach. Simvastatin (SIM), a HMG-CoA reductase inhibitor, showed a remarkable cardioprotective effect in view of its anti-oxidant and anti-inflammatory properties. Here, we investigated the cardioprotective role of SIM on DOX-induced acute cardiotoxicity in an *in vitro* model.

METHODS

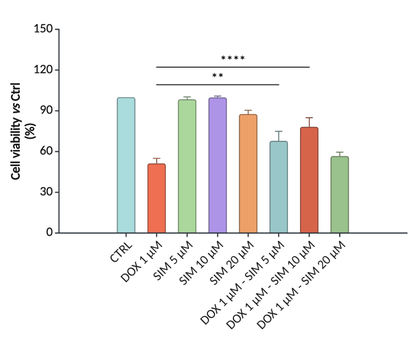
Human cardiomyocytes (HCM) were pre-treated with SIM (5,10,20 μM) for 4h and then DOX (1 μM) was added. The effects of SIM-DOX co-treatment on DOX-induced cardiotoxicity were evaluated at different time points by MTS, microscopy, FACS analysis and Real-Time PCR.

Simvastatin and Doxorubicin co-treatment
***In vitro* effects analyses**

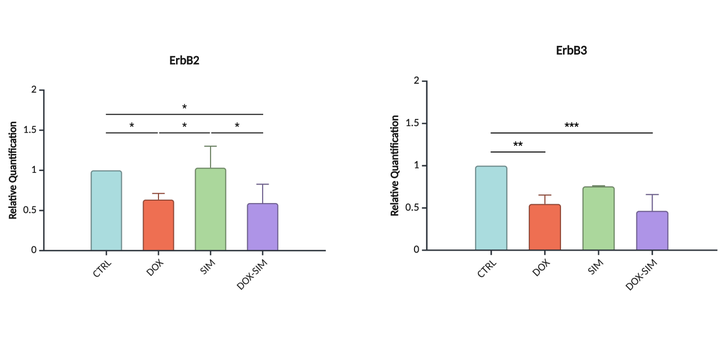


RESULTS

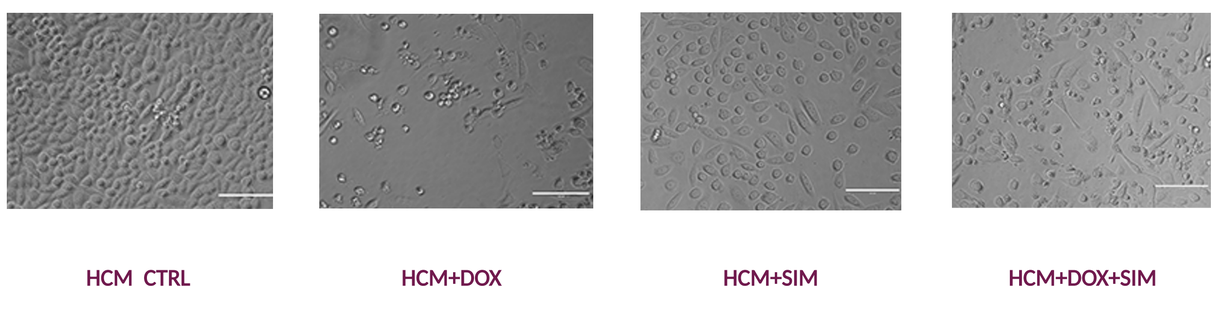
MTS assay results demonstrated that SIM, in co-treatment, significantly ($p<0.01$) reduced DOX-induced cell death after 48 hours. Real time-PCR results reported a significantly ($p<0.05$) decrease of ErbB2 and ErbB3 expression after both DOX alone and in co-treatment. FACS analyses showed that co-treatment significantly reduced the apoptotic process ($p<0.005$), both cytosolic and mitochondrial DOX-induced ROS production ($p<0.05$), as well as cytochrome c release ($p<0.01$) and mitochondrial membrane depolarization ($p<0.005$). A significant ($p<0.05$) reduction was observed also in DOX-induced Cx43 phosphorylation on Ser368 residues increased levels ($p<0.05$) (data not shown). Furthermore, a significant ($p<0.05$) reduction in DOX-induced SOD2 overexpression has been reported in co-treated cells. HCM treated with DOX showed significant morphological alterations, presenting a greater presence of elongated cells with extensions and cellular debris, with an evident decrease in cell density compared to control cells. HCM co-treated with DOX-SIM present a rounded cell morphology, with a rare co-presence of partially elongated cells and higher cell density.



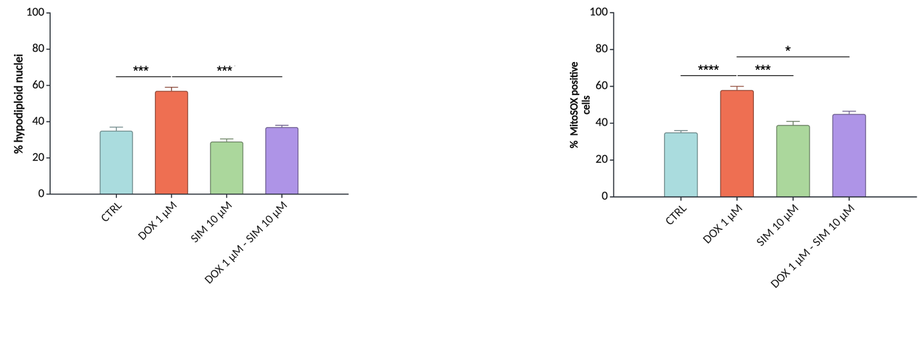
Effects of Doxorubicin, Simvastatin and Doxorubicin-Simvastatin co-treatment on HCM cell line viability as determined by MTS assay after 48 hours treatment. Data are expressed as means ± SD, and analyzed by analysis of variance (ANOVA) followed by Tukey multiple comparisons test. **p < 0.01, ****p < 0.001 Sim + Dox co-treatment.

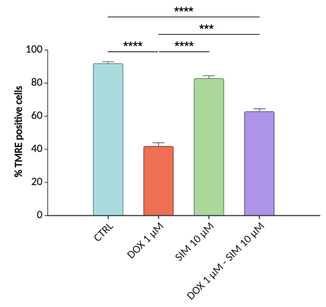


Effects of Doxorubicin (1 μM), Simvastatin (10 μM) and Doxorubicin-Simvastatin co-treatment on ErbB2 and ErbB3 relative gene expression in HCM cell line as determined by Real-Time PCR after 48 hours treatment. Data were calculated using the $2^{-\Delta\Delta Ct}$ method, normalized to GAPDH mRNA levels and then expressed as relative to control (calibrator sample, defined as 1.00). Data are expressed as means ± SD and analyzed by analysis of variance (ANOVA) followed by Tukey multiple comparisons test. *p < 0.05, **p < 0.01, ***p < 0.005.



Effects of Doxorubicin (1 μM), Simvastatin (10 μM) and Doxorubicin-Simvastatin co-treatment on HCM morphology analyzed under a Leica DMi1 inverted microscope using 40x magnification after 48 hours treatments.





Effects of Doxorubicin, Simvastatin, and Doxorubicin-Simvastatin co-treatment on HCM apoptotic process, ROS production, cytochrome c release and mitochondrial membrane depolarization determined by FACS analysis. Data are expressed as mean ± SEM and analyzed by analysis of variance (ANOVA) followed by Bonferroni's test. *p < 0.05, **p < 0.01, ***p < 0.005, ****p < 0.001

CONCLUSIONS

Our results showed that SIM-DOX co-treatment reduces DOX-induced oxidative stress and apoptosis, leading HCM to reduce defense mechanisms and suggesting a possible strategy to protect HCM against DOX-induced damage.

FUTURE DIRECTIONS

Our results will be combined with the epidemiological analysis on cancer patients, with the aim to support for the use of Simvastatin as a cardioprotective agent. Such validation would significantly enhance our understanding and management of DIC, contributing to improved patient outcomes and life expectancy.