Comparison of the inhibition of an OCT3 transporter inhibitor, Nilotinib, on Doxorubicin's effect on cardiac and cancer cell lines

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INTRODUCTION

Doxorubicin (DOX)-induced cardiotoxicity remains a significant barrier limiting its clinical application. A promising new strategy involves targeting how DOX enters cardiac and cancer cells. Research suggests that an OCT3 transporter significantly contributes to DOX entry into heart tissue. By contrast, it is expressed much lower on breast cancer cell lines. Moreover, Nilotinib (NIB) can suppress OCT3 transporter function by 80%. Therefore, exploring the impact of NIB in altering DOX's intracellular accumulation and effects on cardiac and cancer cell lines is an avenue worth exploring.

Objective

- 1) Establish a dose-response curve of DOX and NIB alone to assess their individual effects on cell viability.
- 2) Investigate the impact of NIB on DOX entry within cardiac myoblasts (H9C2) and breast cancer cells (MCF7) to assess if NIB can exert cardioprotective effects while maintaining DOX's anticancer effect.

METHOD

Experiments:

H9C2 myoblast and MCF7 breast cancer cells were seeded in 96-well black plates.

To achieve our first objective, cells were treated with only DOX or NIB to establish a dose-response curve.

To achieve our second objective, NIB was combined with DOX at various titrated combinations using NIB (10 nM, 50 nM, 100 nM, 500 nM, 1 μM, 2 μM, 5 μM) and DOX (10 μM and 40μM). Cotreatment consisted of adding Nib and DOX simultaneously. Pretreatment consisted of adding Nib 24 hours before introducing DOX.

Bioassays were conducted after cells were treated for 24 hours. Intracellular DOX fluorescence intensity was measured at 488/590 nm by Fluoroskan. During data analysis, background fluorescence was controlled by subtracting the intensity of untreated cells from the mean of each Dox/Nib combination. A ratio relative to the CCK was performed to measure the average concentration of Dox within living cells. Finally a ratio relative to the Dox 10 μM and 40 μM control was used to measure the impact of Nib on DOX entry into cells.

Subsequently, cell viability was detected by measuring absorbance at 450 nm after adding a cell counting reagent. A ratio relative to untreated or DOX cells was used to reduce variation caused by seeded cell densities among different experiments.

Statistics:

Data was expressed as a mean ±SE. The statistical significance was analyzed either by t test for two groups or by ANOVA for more than two groups. Values of $p < 0.05$ (*) were considered statistically significant between H9C2 and MCF7 cells under the same DOX/NIB concentrations

RESULTS & DISCUSSION

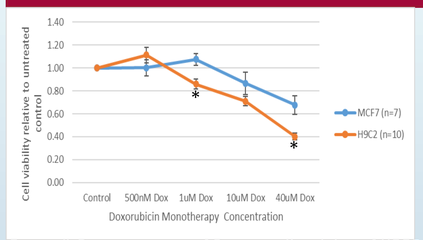

Figure 1) Dose response of Dox on cell viability of H9C2 and MCF7 cells. * = p<0.05

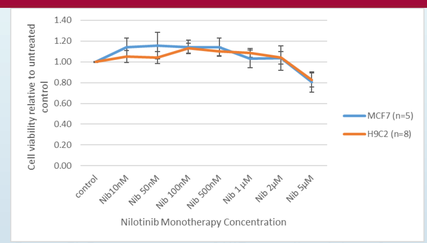

Figure 2) Dose response of NIB on cell viability of H9C2 and MCF7 cells

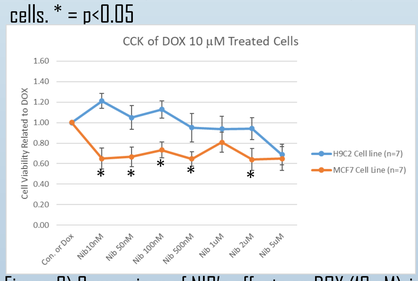

Figure 3) Comparison of NIB's effects on DOX (10 μM)-induced cell damage between H9C2 and MCF7 cells.

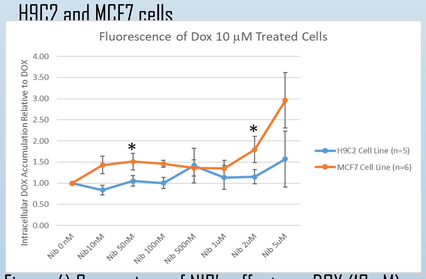

Figure 4) Comparison of NIB's effects on DOX (10 μM) intracellular accumulation between H9C2 and MCF7 cells.

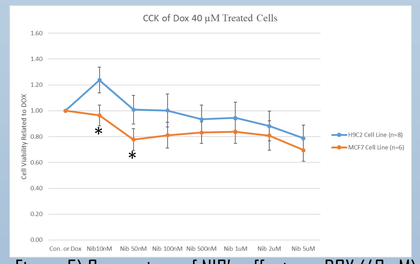

Figure 5) Comparison of NIB's effects on DOX (40 μM)-induced cell damage between H9C2 and MCF7 cells.

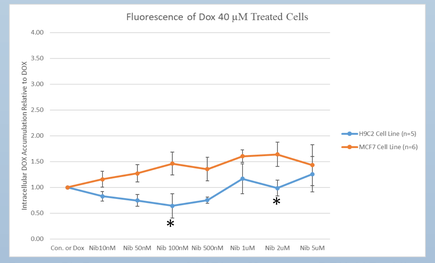

Figure 6) Comparison of NIB's effects on DOX (40 μM) intracellular accumulation between H9C2 and MCF7 cells.

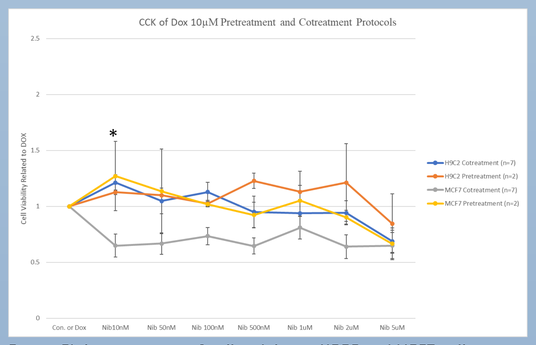

Figure 6) A comparison of cell viability in H9C2 and MCF7 cells under cotreatment or pretreatment regimens using Nib and Dox 10 μM

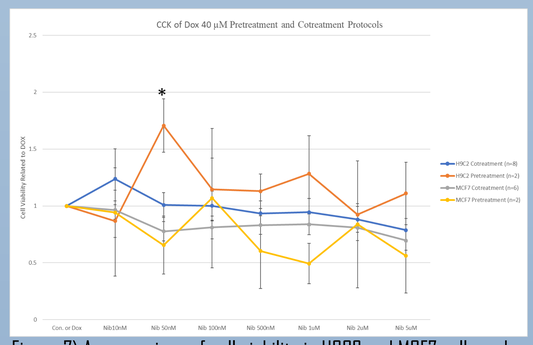

Figure 7) A comparison of cell viability in H9C2 and MCF7 cells under cotreatment or pretreatment regimens using Nib and Dox 40 μM

CONCLUSION

DOX-induced damage was more potent in H9c2 cells than in MCF7 cells. By contrast, NIB (10 nM- 5 μM) slightly affected cell viability in H9c2 and MCF cells.

Lower doses of NIB (10 nM) cotreatment exerted a mild protective effect on H9c2 cells by increasing cell viability to 1.05 ± 0.12 (n=7) and 1.24 ± 0.10 (n=8) when compared to DOX 10 μM and 40 μM respectively. The effects were accompanied by mild to moderate reduction of intracellular DOX accumulation.

Additionally, NIB cotreatment increased DOX's anti-cancer effects on MCF7 cells by reducing cell viability to 0.64 ± 0.11 (NIB 10 nM; n=7) and 0.7 ± 0.09 (NIB 5 μM; n=7) when compared to DOX 10 μM and 40 μM respectively. The effects were accompanied by higher intracellular DOX accumulation.

In conclusion, NIB cotreatment only exerted mild protection of H9C2 cells against DOX with mild to moderate reduction of intracellular DOX accumulation. By contrast, NIB cotreatment potentiated DOX's anti-cancer effects on MCF7 cells with higher intracellular DOX accumulation. More experiments are needed for NIB pretreatment's effects on H9c2 and MCF7 cells.

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