The effects of mitoquinone pretreatment on doxorubicin-induced acute cardiac dysfunction

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INTRODUCTION

Background

Doxorubicin (DOX) is widely used to treat solid tumors and hematological malignancies in children and adults. Although it is an effective anti-tumor drug, DOX causes cumulative and dose-dependent cardiotoxicity, leading to severe cardiomyopathy. One of the proposed principal mechanisms of DOX cardiotoxicity is an increase in reactive oxygen species (ROS) leading to an increase in oxidative stress, which induces toxic damage to the mitochondria of cardiomyocytes. Current preventive strategies include:

- Limiting the cumulative dose to <450 mg/m²
- Antioxidants (e.g., Probucol, Dexrazoxane)
- Anthracycline analogues (e.g., Epirubicin)
- Alternative infusion methods (rapid vs. slow)

Although there are strategies in place, present treatment does not improve prognosis and most management is supportive to alleviate symptoms. Mitoquinone (MitoQ) is an antioxidant agent that specifically targets mitochondria to prevent it from overproducing ROS. It is stored in vivo to prevent and protect cellular damage induced by oxidative stress. Our lab recently determined that MitoQ given as a pre-treatment to DOX mitigates DOX-induced damage and continued to study the mechanisms involved in MitoQ cellular protection in H9c2 cells. This study further investigates MitoQ effects on DOX-induced damage, but on isolated rat hearts.

Objectives

1. To evaluate the effects of DOX and MitoQ on cardiac function of isolated rat hearts.
2. To assess the benefits of MitoQ pretreatment on DOX-induced cardiac dysfunction.

Hypothesis

We hypothesize that MitoQ pre-treatment would show cardioprotective effects on isolated rat hearts.

METHODS

Male SD Rats

- Sprague-Dawley rats weighing between 275-325 g were anesthetized using a mixture of ketamine (87.5µg/g body weight), xyalzine (12.5µg/g body weight) and heparin (1000 U).

Treatment Conditions

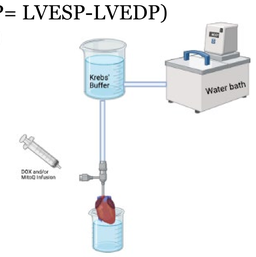
- DOX infusion 60 min.
  - 20 µM
  - 25 µM
- MitoQ infusion 60 min.
  - 0.1-0.5 µM
  - 1-5 µM
- DOX (25 µM) + MitoQ Pre-treatment 10-15 min.
  - MitoQ 0.25-0.5 µM
  - MitoQ 1-2.5 µM

Cardiac Function Parameters

- A pressure inducer was placed into the left ventricle of the rat heart and the following parameters were measured every 5 min. once stable cardiac functions were measured:
  - Left Ventricle End-Systolic Pressure (LVESP)
  - Left Ventricle End-Diastolic Pressure (LVEDP)
  - Left Ventricular Developed Pressure (LVDP= LVESP-LVEDP)
  - Maximal Rate of Rise of LVP (dP/dt (max))
  - Heart Rate (HR)

Langendorff Heart Preparation

- The rat heart was immediately removed after sacrifice and attached to Langendorff Heart Apparatus.
- The isolated heart was then retrogradely perfused with Krebs' buffer at a constant pressure of 80 mmHg at 37 ºC and pH of 7.35-7.45.



Langendorff Heart Preparation

Statistical Analysis

All values are presented as a mean ±SE. Data was analyzed using ANOVA. Values of $p < 0.05$ were considered statistically significant.

RESULTS

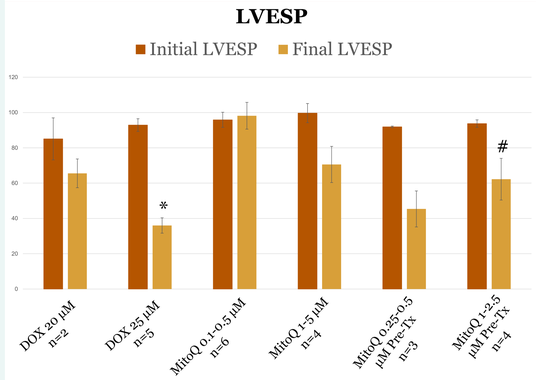


Figure 1: DOX 25 µM infusion (n=5) exhibited significantly lower final LVESP compared to its initial. MitoQ 1-2.5 µM pre-treatment (n=4) significantly improved the LVESP when compared to the final of DOX 25 µM.

(*: $p < 0.05$ vs. initial of treatment group, #: $p < 0.05$ vs. DOX 25 µM)

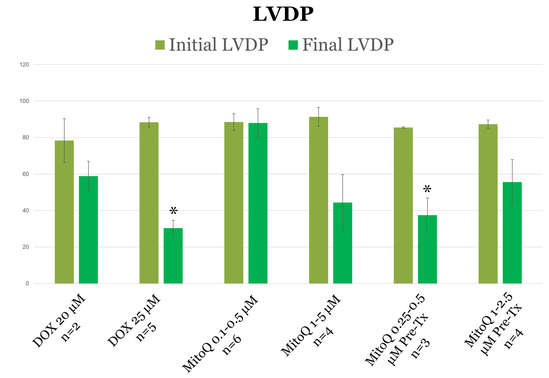


Figure 2: DOX 25 µM (n=5) exhibited significantly lower LVDP compared to its initial. MitoQ 1-2.5 µM pre-treatment (n=4) did not significantly change the LVDP.

(*: $p < 0.05$ vs. initial treatment group)

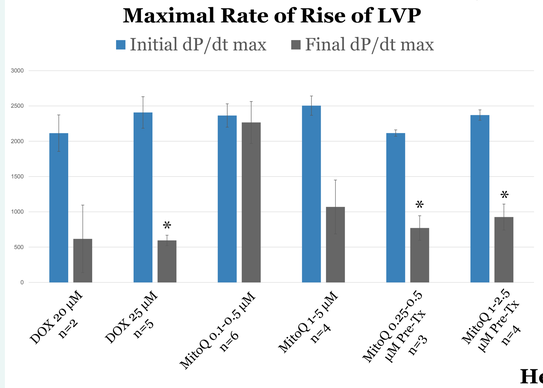


Figure 3: DOX 25 µM (n=5), MitoQ 0.25-0.5 µM pre-treatment (n=3), and MitoQ 1-2.5 µM pre-treatment (n=4) all had significantly lower final LVP compared to their initial.

(*: $p < 0.05$ vs. initial treatment group)

Figure 4: HR was significantly reduced in all treatments involving MitoQ.

(*: $p < 0.05$ vs. initial treatment group)

SUMMARY

Acute infusion of DOX into the isolated hearts dose-dependently reduced some cardiac parameters. Higher dose DOX (25 µM, n=5) induced a higher reduction in the ratios of LVESP, LVDP, and dP/dt(max) to 0.39±0.05, 0.35±0.06, and 0.26±0.05 than those of lower dose DOX infusion (20 µM, n=2; 0.77±0.01, 0.75±0.01, and 0.57±0.01), respectively. DOX at both concentrations had no effect on HR.

Infusion of lower doses of MitoQ (0.1-0.5 µM, n=6) for 60 min. only significantly reduced HR to a ratio of 0.77±0.01 without suppressing other cardiac parameters. By contrast, higher doses of MitoQ (1-5 µM, n=4) that were perfused for 60 min. reduced the ratios of LVESP, LVDP, dP/dt(max), and HR to 0.72±0.12, 0.51±0.18, and 0.45±0.17 0.65±0.07, respectively, with HR being significant.

MitoQ given as pre-treatment for 10-15 min. before DOX 25 µM exhibited better cardiac function accompanied by reduced HR than DOX alone. The improvement is in a dose-dependent manner. In specific, pre-treatment of the higher dose of MitoQ (1-2.5 µM) showed statistically higher LVESP (62.23 mmHg) when compared to the final LVESP of DOX 25 µM alone (36.00 mmHg, $p < 0.05$).

CONCLUSION

1. Infusion of DOX into the heart acutely attenuated cardiac systolic function.
2. MitoQ pre-treatment for 10-15 min. mitigated DOX-induced heart dysfunction.
3. MitoQ significantly reduced heart rate.

Future Studies

The effects and the mechanism of higher doses of MitoQ on the cardiomyocyte mitochondrial function, heart rate, and contractility should be further studied.

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