The Effects of Mitoquinone on Simulated Ischemia/Reperfusion Injuries in H9c2 cells.

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INTRODUCTION

Myocardial infarctions are the leading cause of death in the United States, thus there is a growing need to understand the key mechanisms involved in the treatment of these diseases. Myocardial infarctions occur when decreased blood flow to the myocardium deprives the cardiomyocytes of nutrients. Prompt treatment to restore blood flow after a period of ischemia is required in these cases; however, reperfusion to an ischemic myocardium could result in further damage termed, myocardial ischemia/reperfusion (I/R) injury. I/R injury occurs through the generation of reactive oxygen species (ROS), mainly in the mitochondria, which increases the death of cardiomyocytes.The excess ROS disrupts the function of the mitochondria leading to a decreased production of adenosine triphosphate (ATP) production through the electron transport chain complexes and thus play a vital role in the pathogenesis in I/R injuries.

Mitoquinone (MitoQ), an mitochondria targeted antioxidant, is a ubiquinone derivative that targets the mitochondria through its attachment to triphenylphosphonium cation. Due to the high electrical membrane potential of the mitochondria, the positively charged ion can accumulate inside the mitochondria where it is then converted into its reduced form by the respiratory chain to prevent oxidative harm to the mitochondria (Kong et al., 2021). Therefore, the use of MitoQ represents a promising approach for the treatment and prevention of I/R injuries.

Hypothesis

We hypothesized that H9c2 myoblast cells would be damaged by simulated I/R. Moreover, MitoQ would attenuate myocardial injury, characterized by increased cell viability, compared to non-treated control.

METHOD

The H9c2 myoblast cells (less than 20 passages) were treated with or without various concentrations of MitoQ (0.005, 0.05, 0.1, 0.5, 1, 2, 5 μM) under 3 different mediums: normal (containing 4.5 g glucose and pyruvate), low glucose (containing 1 g glucose and pyruvate), and no glucose/pyruvate medium. The different medium conditions simulated various degree of nutrient deprivation under I/R. Cell viability was evaluated when cells were treated by MitoQ with or without simulated ischemia or I/R (Figure 1 and 2).
Data was expressed as ratio to CCK values of non-MitoQ treated controls and summarized as mean ±SE. The statistic significance was analyzed by ttest for two groups or ANOVA for more than 2 groups. Values of $p < 0.05$ were considered statistically significant.

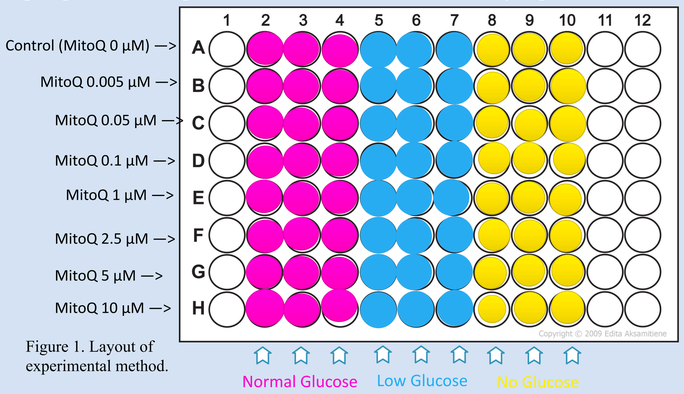


Figure 1. Layout of experimental method.

METHOD

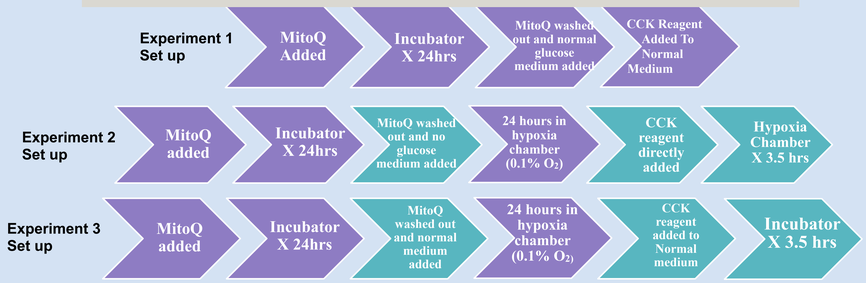


Figure 2. Flow chart of the 3 different experiment set-ups.

RESULTS

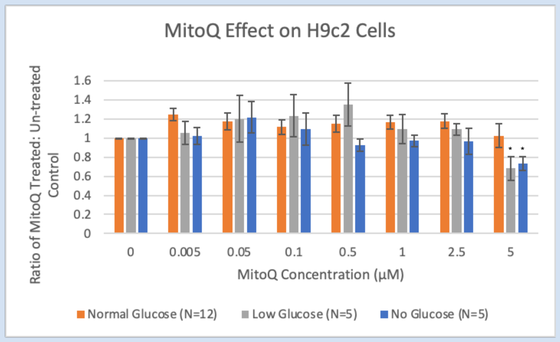


Figure 3. Dose-dependent effects of MitoQ on cell viability under different medium conditions. Low concentrations of MitoQ alone slightly increased cell viability in all three mediums when compared to the non-treated H9c2 cells. Interestingly, MitoQ at 5 μM showed a significant reduction in cell viability in low glucose (0.68 ± 0.12, n =5, $p<0.05$) and no glucose medium (0.74 ± 0.07, n=5, $p<0.05$) when compared to the normal medium (1.03± 0.12, n =12).

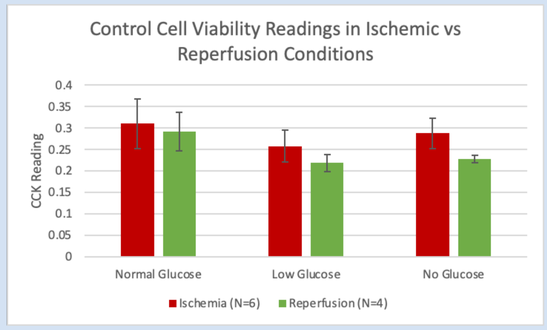
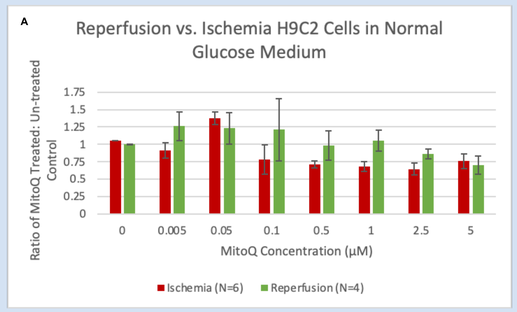
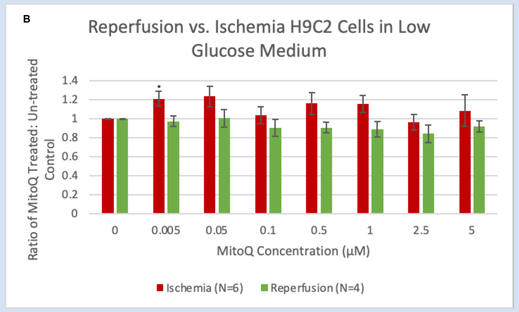


Figure 4. The comparison of CCK of untreated controls under ischemia with/without reperfusion. Compared to the ischemic condition, the cell viability of non-treated H9c2 cells (control) was reduced after the reperfusion particularly in the low glucose and no glucose medium.





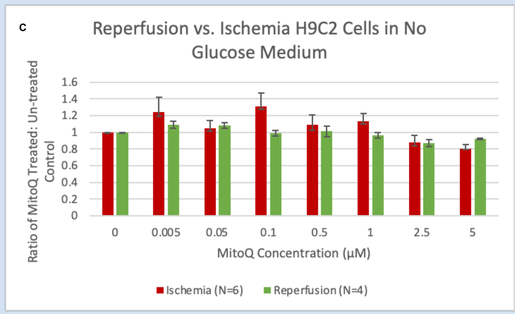


Figure 5A-C. MitoQ showed cardioprotective effects under simulated ischemia in low glucose medium and no glucose medium compared to reperfusion conditions. Particularly, MitoQ at 0.005 μM showed a significant increase in cell viability in low glucose (1.21 ± 0.07, N =6, $p<0.05$) when compared to non-treated control in ischemic conditions. Under simulated reperfusion conditions, there was only slight increase in cell viability in low glucose and no glucose mediums.

CONCLUSION

Dose dependent effects of MitoQ can be modified by the cell media. The higher MitoQ (e.g. 5 uM) reduces cell viability when the medium contains low glucose or no glucose.

Simulated reperfusion conditions reduces cell viability compared to ischemic conditions. The reduction is greater in cells with medium containing low glucose or no glucose than medium containing normal glucose.

MitoQ pretreatment shows slight cardioprotective effects against ischemia and reperfusion in cells with normal medium. By contrast, MitoQ pretreatment only shows slight mitigation against ischemia, not reperfusion, in cells with medium containing low or no glucose.

Further studies can be conducted by measuring the intracellular ROS production and mitochondrial membrane potential under ischemia and reperfusion conditions with and without MitoQ pretreatment.

REFERENCES

- Kong, L., Li, X., Li, Y., Zhang, Y., & Han, X. (2021). Emerging roles of circular RNAs in heart failure. Heart Failure Reviews, 26(2), 255-266. https://doi.org/10.1007/s10741-020-10028-4
- Kuznetsov, A. V., Javadov, S., Sickinger, S., Frotschnig, S., & Grimm, M. (2018). H9c2 and HL-1 cells demonstrate distinct features of energy metabolism, mitochondrial function and sensitivity to hypoxia-reoxygenation. Frontiers in Physiology, 9, 1130. https://doi.org/10.3389/fphys.2018.01130

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