Role of ATPase Inhibitory Factor 1 in Preventing Doxorubicin Induced Cardiotoxicity

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Introduction

Doxorubicin (Dox) has been widely used as a chemotherapeutic agent for treating various cancers. However, despite its efficacy its clinical use is limited due to its severe cardiotoxic effects. The primary mechanism underlying this toxicity involves mitochondrial dysfunction characterized by impaired mitochondrial membrane potential, reduced ATP production, and increased generation of reactive oxygen species (ROS). Studies have shown the key role of ATPase inhibitory factor 1 (IF1), a mitochondrial protein, in preserving mitochondrial function by stopping ATP hydrolysis and ultimately increasing ATP storage. This study aims to investigate the role of IF1 in mitigating the mitochondrial toxicity induced by Dox in human cardiomyocytes.

Methods

The effects of IF1 overexpression and knockout on mitochondrial function, cell viability, and ROS generation in the presence of Dox was investigated using human cardiomyocyte cells (AC-16). The cells were seeded into one Seahorse XF24 well plate and one Agilent xCELLigence 16 well plate. They were than transduced with vectors for IF1 overexpression (OE), a dominant negative form of IF1 (E55A), and a null vector (GFP) as a viral internal control, all at a multiplicity of infection (MOI) of 75. 24 hours post-transduction, the cells were exposed to 2.5 micromolar of Dox for 24 hours. Cell viability was assessed using the cellular impedence assay, while mitochondrial function was evaluated using the Seahorse XF24 analyzer.

The cell impedance assay measures changes in electrical current by passing a current through gold electrodes on which cells are attached. The presence and behavior of the cells can alter the electrical current, and these changes can be used to assess cell viability.

Figure 1

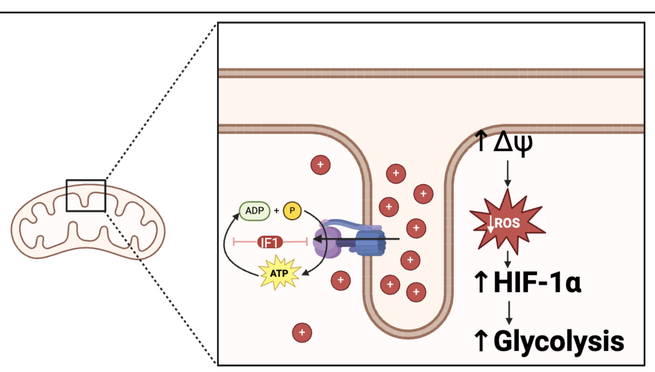

Figure 1- A diagram showing the mechanism of action of IF1 in persevering mitochondrial membrane potential through the inhibition of ATPase activity of ATP synthase.

Figure 2

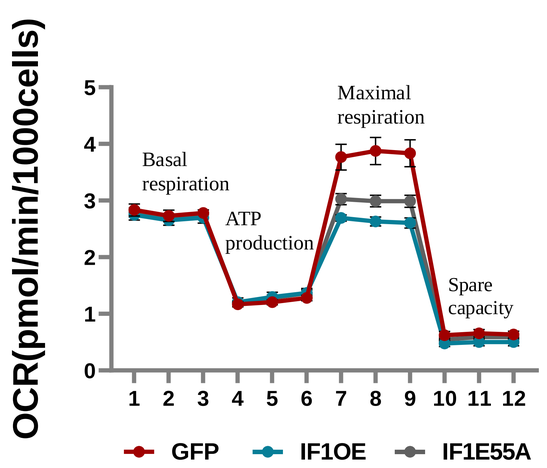

Figure 2- A line graph showing the oxygen consumption rate (OCR) of GFP (red), IF1OE (blue), and IF1E55A (gray) from the Mito Stress test using the Seahorse Analyzer.

Figure 3

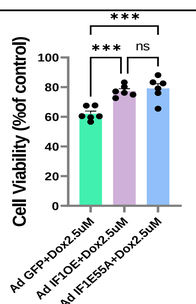

Figure 3A- A bar graph comparing cell viability (% of control) of cells expressing GFP (green), IF1OE (purple), or IF1E55A (blue) after exposure to 2.5 μM doxorubicin (Dox). The cell viability was assessed using an MTT assay. IF1OE and IF1E55A had significantly increased cell viability compared to GFP ($p < 0.05$) after Dox exposure.

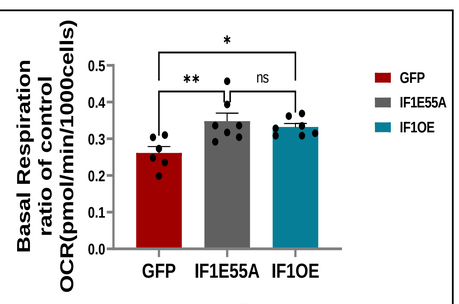

Figure 3B- A bar graph illustrating basal respiration (pmol/min/1000 cells) measured using an XF24-O2 analyzer. Cells expressing GFP (red), IF1E55A (gray), and IF1OE (blue) were compared. IF1E55A and IF1OE show significantly increased ATP production compared to GFP ($p < 0.05$).

Figure 4

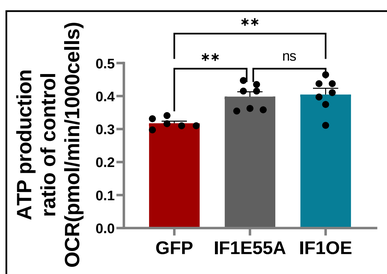

Figure 4A- A bar graph illustrating ATP production rates (pmol/min/1000 cells) measured using an XF24-O2 analyzer. Cells expressing GFP (red), IF1E55A (gray), and IF1OE (blue) were compared. IF1E55A and IF1OE show significantly increased ATP production compared to GFP ($p < 0.05$).

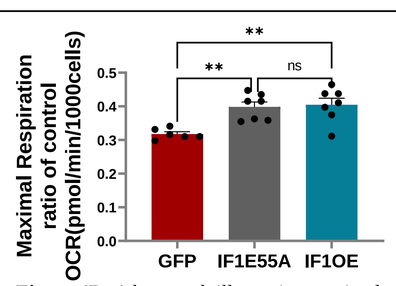

Figure 4B- A bar graph illustrating maximal respiration (pmol/min/1000 cells) measured using an XF24-O2 analyzer. Cells expressing GFP (red), IF1E55A (gray), and IF1OE (blue) were compared. IF1E55A and IF1OE show significantly increased maximal respiration compared to GFP ($p < 0.05$).

Figure 5

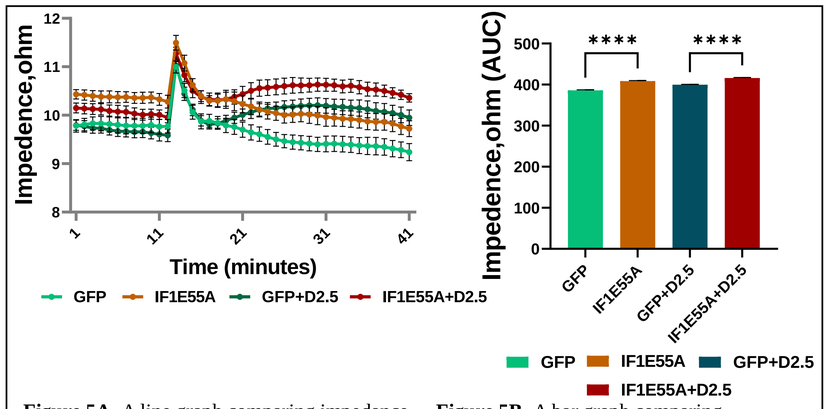

Figure 5A- A line graph comparing impedance (ohm) of cells expressing GFP or IF1E55A, with or without exposure to 2.5 μM doxorubicin. The control groups are GFP (green) and IF1E55A (orange) while the groups exposed to Dox are GFP+Dox (blue) and IF1E55A+Dox (red). IF1E55A has a significantly increased impedance compared to GFP ($p < 0.05$) and this relationship was not affected by doxorubicin exposure.

Figure 5B- A bar graph comparing impedance (ohm) of cells expressing GFP or IF1E55A, with or without exposure to 2.5 μM doxorubicin. The control groups are GFP (green) and IF1E55A (orange) while the groups exposed to Dox are GFP+Dox (blue) and IF1E55A+Dox (red). IF1E55A has a significantly increased impedance compared to GFP ($p < 0.05$) and this relationship was not affected by doxorubicin exposure.

Results

Statistical analysis revealed a significant difference ($p<0.05$) in cell viability, ATP production, and maximal respiration between the IF1OE and IFE55A groups compared to the GFP group. As shown in Figure 4A, IF1OE and IFE55A both markedly increased ATP production relative to GFP, suggesting they play a key role in preserving mitochondrial membrane potential. Furthermore, they both exhibited significantly ($p<0.05$) higher maximal respiration (figure 4B), and basal respiration (figure3B), which is indicative of enhanced mitochondrial function, potentially contributing to improved cardiovascular health and longevity.

Conclusion

Both IF1OE and IF1E55A promoted increased cell viability, although through distinct mechanisms. IF1OE enhanced glycolysis to support cell survival, whereas IF1E55A preserved mitochondrial membrane potential by allowing ATPase to function normally. These differential pathways suggest that both forms may offer protection against mitochondrial toxicity through different metabolic changes.
The findings of this study are consistent with previous research, further supporting the role of IF1 in mitochondrial function. Future studies can use this model to investigate the potential therapeutic effects of compounds such as kynurenic acid on preserving cardiovascular integrity by modulating IF1 activity as well as the long-term differences between the two groups. This approach may provide new insights into treatments aimed at mitigating mitochondrial dysfunction and promoting cardiovascular health in patients undergoing doxorubicin therapy.