Investigating Methylglyoxal Mediated DNA Damage in Pulmonary Arterial Hypertension

Anna Park and Kurt W. Prins, MD, PhD
Cardiovascular Division, Department of Internal Medicine, University of Minnesota, Minneapolis, MN

Abstract

Introduction: Pulmonary arterial hypertension, PAH, is a progressive disorder characterized by an increase in blood pressure in the pulmonary arteries.[1] The hypertensive force driving PAH often results in detrimental physiological changes of the pulmonary arteries and right ventricle (RV), ultimately leading to RV failure and death.[1] A key indicator of PAH in patients is mitochondrial dysfunction, and the resulting presence of methyl glyoxal (MG): a toxic intermediate byproduct of glycolysis.[2] While little is understood of the process, MG intermediates are thought to be able to induce post-translational DNA damage.

Methods: Western blot analysis and immunohistochemistry were used to establish a base relationship between MG and DNA damage. The results were quantified to examine the extent of DNA damage in H9C2 cardiomyoblasts. An immunohistochemistry (IHC) was used to visualize and further quantify MG mediated DNA damage.

Results: From the western blots, MG expression increased 87.34 fold in cardiomyblasts exposed to 625µM MG for 4 hours and was borderline significant with a p value of 0.217. pH2AX expression increased significantly by 9.99 fold in the same cells with a p value of 0.041. The IHC revealed a positive relationship between MG and DNA damage in cardiomyoblasts.

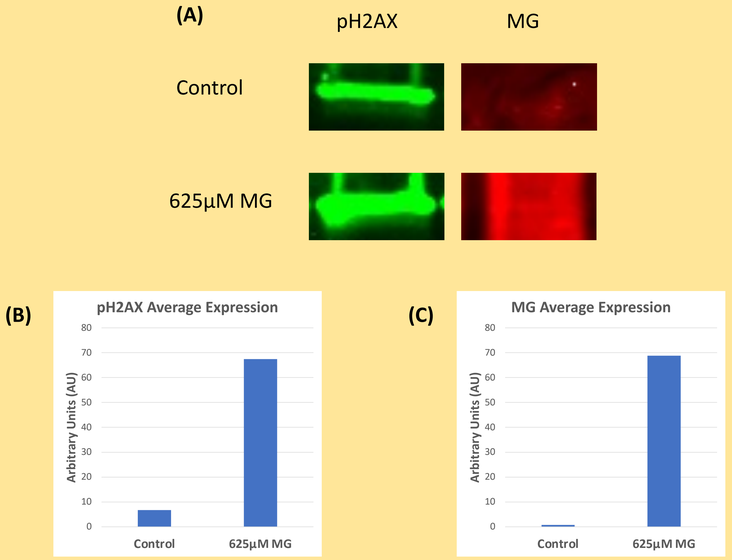


Figure 1: Quantification of H9C2 cell western blot under control and 625µM MG conditions. (A) Representative western blot of H9C2 cells with pH2AX antibodies and MG antibodies. (B) Quantitative graphical results of the average expression of pH2AX antibodies in the control and the 625µM MG groups. (C) Quantitative graphical results of the average expression of MG antibodies in the control and the 625µM MG groups.

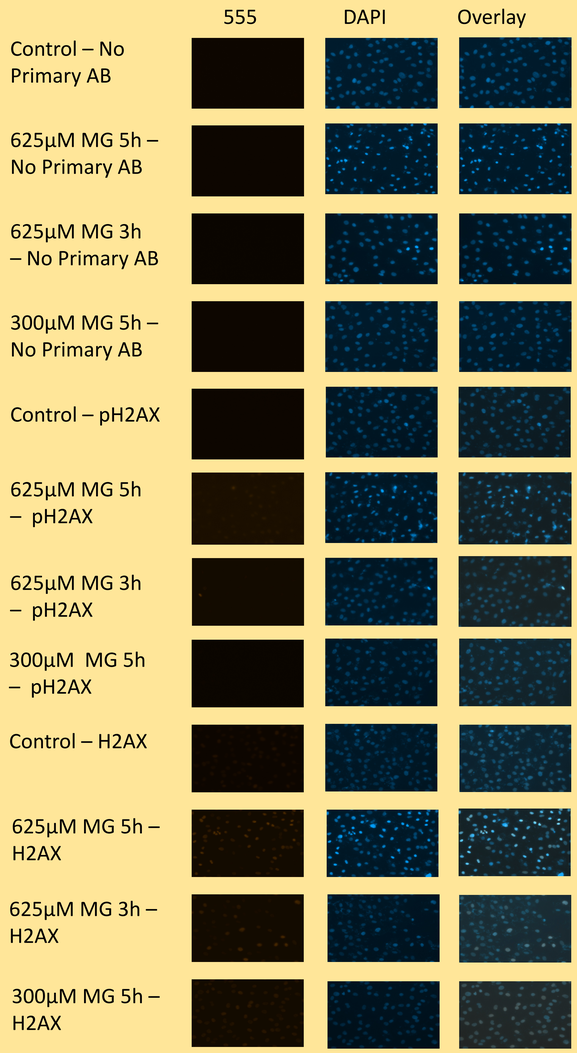
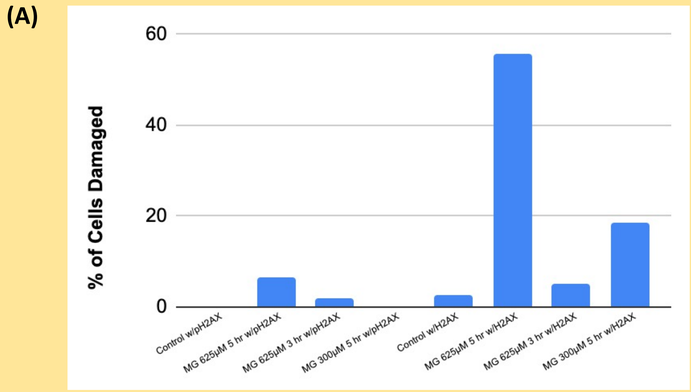


Figure 2: Immunohistochemistry results of cells treated with varying amounts of MG for different amounts of time at 20X magnification. Cells with MG mediated DNA damage tagged with antibodies H2AX and pH2AX visualized in the 555 cm^{-1} wavelength channel (left column). DAPI staining of cells present visualized in the 400 cm^{-1} wavelength channel (middle column). Overlayed image showing both channels (right column).



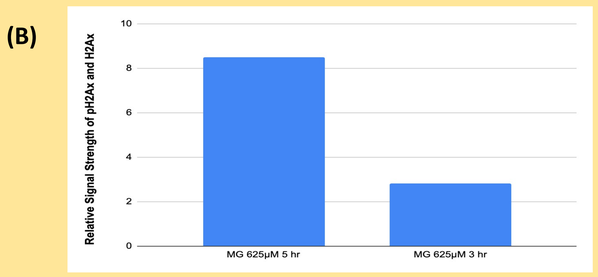


Figure 3: Graphical Analysis of IHC Cell DNA Damage Results. (A) Average percent of damaged cells in each group with a positive trend of MG exposure and percent of cells experiencing DNA damage. (B) The relative strength of the H2AX and pH2AX antibodies and subsequent signals detected, with H2AX displaying a stronger signal than pH2AX.

Conclusions: An increased amount of MG is a strong indicator of PAH. Cardiomyoblast cells exposed to MG during the differentiation stage displayed a significant increase in DNA damage ($p<0.05$). Thus, there is a relationship between the amount of MG cells are exposed to and DNA damage.

Future Directions: Further research could examine the long-term effects of heightened MG exposure in cardiomyoblast cells. Research could also investigate the more precise relationship between excess MG and right ventricle dysfunction. Additionally, the DJ-1 enzyme and its ability to reverse DNA damage caused by MG could be explored.

References:

1. Ryan, J. J., & Archer, S. L. (2014). The right ventricle in pulmonary arterial hypertension: disorders of metabolism, angiogenesis and adrenergic signaling in right ventricular failure. *Circulation research*, *115*(1), 176–188. https://doi.org/10.1161/CIRCRESAHA.113.301129
2. Champion, H., et. al. (2009). Comprehensive Invasive and Noninvasive Approach to the Right Ventricle–Pulmonary Circulation Unit. *Circulation, 120*(11), 992-1007. https://doi.org/10.1161/CIRCULATION. Circulation. 2009;120:992–1007