Synergistic Effects of Methylglyoxal and Hyperglycemia on ROS generation and the viability of cultured H9c2 Myoblast Cells

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Introduction

Diabetes mellitus is a disease characterized by abnormally high blood glucose levels (fasting levels >7mmol/l). It has been found to be closely associated with increased incidence of heart failure without hypertension, coronary artery disease, and other comorbidities (1). The deleterious effects of diabetic conditions in the heart hasn't been fully understood. This study will focus on the possible synergistic effects of glucose and methylglyoxal on cardiomyocytes.

A principal condition of diabetes is hyperglycemia which leads to increased intracellular levels of glucose metabolism associated with formation of mitochondrial reactive oxygen species (ROS) (2). Chronic complications stem from two main processes: nonenzymatic glycation and osmotic damage (Figure 1). Another principal condition of diabetes is increased methylglyoxal levels (2). Methylglyoxal is a highly reactive carbonyl, and is considered highly cytotoxic to the body (Figure 2). It has the ability to modify other proteins which can lead to the development of advanced glycation endproducts (AGE) which are involved in the pathogenesis of vascular problems in conditions such as diabetes (3).

Literature has suggested the synergistic effects of high glucose and methylglyoxal have adverse effects on cell viability, ROS generation, and ATP production in human mononuclear cells, human umbilical vein endothelial cells and endothelial progenitor cells, yet the effects remain to be elucidated in H9c2 Myoblasts.

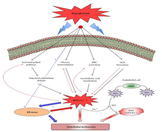

Figure 1: Cellular response to elevated glucose levels (4)

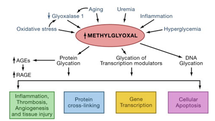

Figure 2: Cellular response to elevated methylglyoxal levels (5)

Hypothesis

We hypothesis that the synergistic effects of glucose (HG) and methylglyoxal (MG) would change oxidative stress, and possibly induce cell death as compared to individual treatments of each compound. Additionally, we will further test if metformin (Met) can decrease MG/HG individually or co-treatment induced cell malfunction.

Methods

H9c2 Cell Preparation: Rat H9c2 were obtained from American Type Culture Collection (ATCC, CRL-1446). Collection cells were maintained in 75 cm^2 flasks and petri dishes using 4.5 g/mL glucose Dulbecco's Modified Eagle Medium (DMEM, Corning) with 10% fetal bovine serum (FBS, Corning) and 1% penicillin streptomycin solution (Corning) at 37° C and 5% CO_2 in an incubator. Once the cells are at 70-80% confluence, the cells were harvested using trypsin-EDTA (Sigma-Aldrich) (3 mL/flask and 2 mL/ dish). Cell Density was counted using trypan blue .3% kit (Sigma-Aldrich) and seeded into 96-well plates to obtain 2-3 x 10^4 cell/well

Measurement of H9c2 cell viability after HG/MG/Met Treatment: H9c2 rat myoblasts were subjected to different doses of glucose and/or methylglyoxal and/or metformin and incubated for 24 hours to evaluate the dose-response effects on cell viability. Cell viability was determined by measuring absorbance at 450 nm after adding tetrazolium as instructed by CCK8 kit (Dojindo Molecular Technologies, Inc).

Methods

Measurement of H9c2 ROS generation after HG/MG/Met Treatment: H9c2 rat myoblasts were incubated with 25 μM non-fluorescent and cell permeable dichlorofluorescein diacetate (DCFDA) for 45 minutes. After washing out DCFDA from incubation buffer, cells were subjected to incubation of different doses of glucose and/or methylglyoxal and/or metformin. After 24 hours of treatments, intracellular ROS generation oxidizes DCFDA to a fluorescent DCF and was measured at excitation of 480 nm, and emission at 520 nm by using Fluroskan Ascent CF scanner (Thermo Scientific, Inc).

Statistical Analysis: All experiments were performed in triplicate and repeated at least three times. All values are presented as a mean ± SEM. Data was analyzed using one-way ANOVA followed by Student-Newmann-Kewls post hoc. Values of p<0.05 were considered statistically significant.

Results

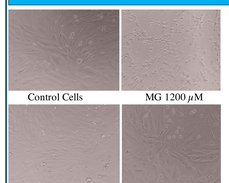

Figure 3: pictures of H9c2 cells after different treatments for 24 hours(10x). Compared to control cell, HG (40 mM) did not cause visible cell damage. By contrast, MG (1200 μM) showed obvious cell death, whereas Met (20 mM) protected the cells from MG-induced cell death .

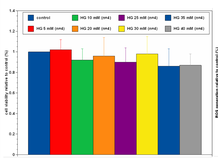

Figure 4a: Effects of glucose (HG) on cell viability. There was no statistical difference of cell viability between the control group and glucose groups (5 mM-40 mM).

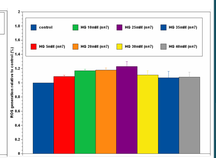

Figure 4b: Effects of glucose (HG) on ROS generation. There was no statistical difference of ROS generation between the control group and the glucose group (5 mM-40 mM).

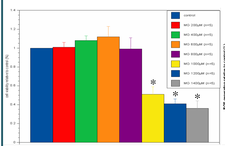

Figure 5a: Effects of methylglyoxal (MG) on cell viability. Higher methylglyoxal significantly reduced cell viability at doses of 1000 μM (51 ± 8%), 1200 μM (41 ± 5%), and 1400 μM (36 ± 8%) compared to control cells. *p < 0.05 vs. control

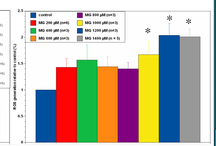

Figure 5B: Effects of methylglyoxal (MG) on ROS generation. Higher methylglyoxal significantly increased ROS generation at doses of 1000 μM (167 ± 27%); 1200 μM (204 ± 22%); and 1400 μM (201 ± 15%) compared to control cells. *p <0.05 vs. control

Results

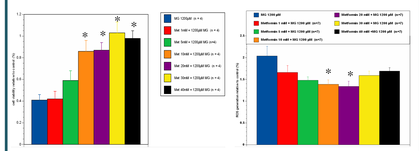

Figure 6a: Effects of Metformin (1 mM-40 mM) on methylglyoxal (MG 1200 μM) caused cell death. Metformin doses (10 mM-40 mM) significantly increased cell viability compared to methylglyoxal. *p<0.05 compared to methylglyoxal.

Figure 6b: Effects of Metformin (1 mM-40 mM) on methylglyoxal (MG 1200 μM) caused ROS generation. Metformin (10 mM – 20 mM) significantly decreased ROS generation compared to methylglyoxal. *p<0.05 compared to methylglyoxal

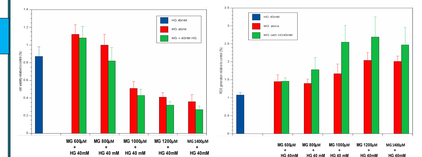

Figure 4: Cotreatment effects of HG (40 mM) + MG (600 μM – 1400μM) on cell viability (n=5). An additive effect was seen in all doses.

Figure 4: Cotreatment effects of HG (40 mM) + MG (600 μM – 1400μM) on ROS generation (n=4). An additive effect was seen in all doses.

Conclusions

The data suggest that higher concentrations of methylglyoxal, not glucose, induces H9c2 cell damage and metformin can protect cells from the methylglyoxal insult possibly by reduction of ROS production. Moreover, hyperglycemia and methylglyoxal tend to induce additive cell damage associated with increased ROS production. Further studies will examine the effect of both compounds on ATP levels, AGE levels, and mechanism of cell death.

References

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Acknowledgements

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