Introduction

Cardiovascular disease is one of the many complications that can arise from diabetes. Diabetic patients may undergo two main cellular changes regardless if glucose concentrations are properly maintained: advanced glycation of end (AGE) products and overwhelming oxidative stress within the cell. Although low levels of AGE are found normally at a basal level within the blood, diabetics have shown to have an increased level that may lead to afterclerosis in vasculature.

Methylglyoxal, a byproduct of glucose metabolism, has been shown to be elevated in the blood of diabetic patients and has been identified as an intermediate in the production of advanced glycation end products. Due to its structure, methylglyoxal is highly reactive with a wide range of cellular components. Specifically in the formation of AGEs, methylglyoxal modifies a select few amino acids and leads to final production of AGES. The consequences of these AGES can cause inflammation, oxidative stress and cell death. Similarly, methylglyoxal is able to produce high levels of reactive oxygen species (ROS) within the cell via mitochondrial modification which results in a loss of membrane potential and an increase in intracellular calcium concentrations. High levels of ROS are damaging to the cell and can lead to alteration in gene expression, inflammatory responses, structural changes, and cell death. The effects and mechanism of methylglyoxal have not been fully elucidated.

In order to reverse the effects of methylglyoxal, our study tests three compounds that have either been utilized in the treatment of diabetes or have been shown to decrease the amount of AGES in the cell. Metformin, a common management medication for diabetes, works by lowering the level of endogenous methylglyoxal in the body, thus decreasing the quantity of AGEs produced and lessening the production of ROS. Aminoguanidine hydrochloride and pyridoxamine dihydrochloride act to reduce levels of AGES by inhibiting one of the intermediate steps, therefore decreasing the effects of AGES and decreasing the amount of ROS as well. Metformin, aminoguanidine hydrochloride, and pyridoxamine dihydrochloride utilize different mechanisms that interfere with the development of AGES in the body, but these protective properties have not been tested with respect to cardiomyocytes.

In this study we compared the effects of metformin, aminoguanidine hydrochloride, or pyridoxamine dihydrochloride on methylglyoxal-induced cell damage and ROS production in H9C2 myoblast cells.

Hypothesis

We hypothesized that methylglyoxal reduced viability of H9C2 myoblast cells in a dose-dependent manner by increasing free radicals. By contrast, concurrently treated with both methylglyoxal and either metformin, aminoguanidine hydrochloride, or pyridoxamine dihydrochloride would increase cell viability accompanied with reduction of free radicals.

Methods

Measurement of cell viability: H9C2 rat myoblasts were seeded at 2x10^4 cells per well 24 hours prior to experimentation. For testing the dose-response of methylglyoxal, cells were treated with varying concentrations (800 µM, 1200 µM, 2000 µM) of methylglyoxal and incubated for 24 hours before analysis. To determine the protective effects, cells were treated with metformin (1–40 mM), aminoguanidine hydrochloride (250 µM–2000 µM) and pyridoxamine dihydrochloride (0.1 µM–15 µM) in presence of methylglyoxal (1200 µM) and monitored for dose-dependent effects after 24 hours. Cell viability was assessed using CCK-8 assay (Dojindo Molecular Technologies) after washing out all the compounds from medium. Moreover, cell morphology was observed by microscopy. Cell viability was expressed as the ratio of CCK readings to the non-treated control.

Measurement of intracellular ROS by DCFDA: After H9C2 rat myoblasts were seeded for 24 hours, cells were replaced with 20 µM non-fluorescent dichlorofluorescein diacetate (DCFDA, Abcam) for 45 minutes to allow the dye to load into the cell. After washing out the unloaded DCFDA, the cells were incubated with media containing 1200 µM methylglyoxal with metformin (1–40mM) or aminoguanidine hydrochloride (250µM-800µM). Inside the cell, DCFDA was deacetylated by cellular esterases to a non-fluorescent compound, which is later oxidized by ROS into a highly fluorescent compound2,7 – dichlorofluorescein (DCF). At 1 hour, 24 hours and >36 hours after treatment, fluorescence was measured at excitation (548 nm) and emission (527 nm) using a Fluoroscan Ascent FLX (Thermo Scientific). The change of ROS was expressed as the ratio to the baseline of non-treated control cells.

Results

Cell Viability (percent of control) (A) Control 400µM 600µM 800µM 1000µM 1200µM 2000µM

We found that higher doses of methylglyoxal reduced cell viability in a dose-dependent manner which may be related to increased levels of ROS within the cell. However, when methylglyoxal was introduced with metformin, aminoguanidine hydrochloride, or pyridoxamine dihydrochloride, cell viability was significantly improved (p<0.05). Meanwhile, higher intracellular ROS caused by methylglyoxal was significantly decreased in the presence of metformin, aminoguanidine hydrochloride. Future studies will investigate mitochondrial function and the possible mechanisms underlying the effects of methylglyoxal, metformin and aminoguanidine hydrochloride.

Conclusions

We hypothesized that methylglyoxal reduced viability of H9C2 myoblast cells in a dose-dependent manner by increasing free radicals. By contrast, concurrently treated with both methylglyoxal and either metformin, aminoguanidine hydrochloride, or pyridoxamine dihydrochloride would increase cell viability accompanied with reduction of free radicals.

References


Acknowledgements

This research was supported by the Division of Research, Department of Bio-Medical Sciences, and Center for Chronic Disorders of Aging at Philadelphia College of Osteopathic Medicine.