Analysis diffusion and glycation rate of artery in high concentration sugar condition via autofluorescence of advanced glycation end productions

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ABSTRACT

Cardiovascular complication is a major health concern for diabetic patients. Elevated blood sugar levels lead to the formation of advanced glycation end products (AGEs), which are implicated in diabetic pathogenesis. In this study, we investigate the effect of prolonged exposure to elevated sugar level by studying the combined effect of diffusion and glycation rate in arteries from different commonly-consumed simple sugars. Since some AGEs are autofluorescent, we will perform multiphoton autofluorescence imaging to quantify the rates of fluorescent AGEs formation in elastic fibers and collagen fibers. Cross-section imaging of arteries and spatial and temporal profiles of autofluorescence of the blood vessel will be investigated.

Keywords: artery, glycation, diffusion, autofluorescence, multiphoton imaging

1. INTRODUCTION

The formation and accumulation of advanced glycation end products (AGEs) contribute to diabetic complications such as retinopathy, neuropathy, nephropathy, and cardiovascular diseases \cite{1,2}. It is clear that the development of effective technique in AGE detection \cite{3} and the establishing the correlation of measured AGE parameters to diabetic pathogenesis are invaluable in the monitoring of disease progression and drug discovery of anti-AGE compounds \cite{1,4}. Since some AGE are fluorescent \cite{5,6}, therefore, we propose to investigate the use of fluorescent AGEs (fAGEs) in tissues to predict the degree of pathogenesis in the cardiovascular system. Specifically, we will study the effects of glucose, fructose, and galactose in the production of fAGE in porcine arteries. At selected periods after sugar treatment, the tissues will image by multiphoton microscopy. The effects of the three sugars on fAGE production in porcine arteries will be characterized.

2. MATERIALS AND METHODS

2.1 Preparation of glycated porcine arteries

Porcine aorta were bought from local market. The diameter of blood vessel tapers from the heart, and branches of the aorta was acquired. In order to avoid effects due to different tissue size, we only used specimens in which the inner diameter of aorta was 10–15 mm, and the outer diameter was 13–20 mm. Each section of the aorta used was 30 mm in length, with no vascular branching. All aorta specimens were washed in 0.01 M phosphate buffered saline (PBS, Sigma-Aldrich, St. Louis, MO, USA), and soaked in 1% povidone-iodine solution for 10 seconds for sterilization. The specimens were then washed again in sterilized PBS solution. All glycated tissues were incubated at 37°C and 5% of CO$_2$ for different periods. The Day 0 control specimen was prepared by washing in 1% povidone-iodine solution and sterilized PBS solution.

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We used three types of glycation solution all of which were simple sugar in type. Specifically, we used glucose, galactose and fructose as they are the most common simple sugars in daily diet. Therefore, soaking porcine aortas in the sugars solutions mimics physiological hyperglycemia condition in diabetes patients. The concentrations of glucose (Sigma-Aldrich, St. Louis, MO), galactose (Sigma-Aldrich, St. Louis, MO) and fructose (Sigma-Aldrich, St. Louis, MO) were all 0.5 M in 0.05M PBS solution with 1% penicillin-streptomycin (Gibco, Grand Island, NY). The control solution was composed of 0.05 M PBS and 1% penicillin-streptomycin. The outer surface and cross-sections of aorta section were coated food grade Teflon spray (Ultragrease TF Spray, Cogelsa, Barcelona, Spain) to prevent sugar molecules from contacting the exterior of aorta tissues. Each section of aorta was incubated in 30 mL glycation solutions which is replaced every four days.

2.2 Multiphoton imaging

Tissues for imaging would be removed from the glycation solution and the middle section of the glycated aorta, with a thickness of about 2 mm, was used. All samples were covered with a 0.17 mm thickness cover glass (22 × 22 mm²) for multiphoton microscopy imaging. Aorta sections would be placed with cross-section under the glass slide, and imaged from inner surface to outer surface.

2.3 Data analysis

Collagen along with elastic fibers are the two main components of the aorta tissue. ImageJ was used for image stitching analysis of the autofluorescence profile. OriginPro® was used to draw the intensity curve.

3. RESULTS AND DISCUSSION

Shown in Figs. 1 and 2 are the autofluorescence and second harmonic generation profiles of the treated aorta sections treated for 17 and 44 days, respectively. As is evident from the images, autofluorescence signal increase with increased treatment time.

![Figure 1. Autofluorescence (red) and second harmonic generation (green) profiles of the treated aorta sections treated for 17.](image-url)
To study the effect of different sugars on treated aortas. The images shown in Figs. 1 and 2 are split into those due to glucose (Fig. 3), galactose (Fig. 4), and fructose (Fig. 5). Also plotted in Figs. 3-5 are the autofluorescence intensity profiles across the aorta sections as a function of position.

Figure 3. A). Autofluorescence (red) and second harmonic generation (green) images of glucose-treated porcine aorta section at Days 0, 17, and 44. B). Autofluorescence intensity profiles across the aorta sections as a function of position.
Figure 4. A). Autofluorescence (red) and second harmonic generation (green) images of galactose-treated porcine aorta section at Days 0, 17, and 44. B). Autofluorescence intensity profiles across the aorta sections as a function of position.
CONCLUSION

In this study, we investigated the effect of three commonly consumed sugar molecules, glucose, galactose, and fructose on porcine aorta. We observed that with prolonged incubation time, there is an increase in autofluorescence, attributable to fluorescent advanced glycation end products. In addition, different simple sugars contribute to different glycation rates in the aorta tissues. With further development, autofluorescence may be an effective way to monitor diabetic pathogenesis.

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