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Multiphoton imaging of autofluorescent advanced glycation end product formation in porcine aorta

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ABSTRACT

Exposure of tissues to sugar lead to the formation of advanced glycation end products (AGEs), contributing to diabetic complications. In human physiology, the vasculature is in direct contact with blood, thus the effect of diabetes is expected to be most severe with the vasculature. In this study, we incubated excised porcine aorta in D-glucose, D-galactose, and D-fructose solutions. Multiphoton microscopy shows that for Days 4 to 48 incubation, autofluorescence is constant along the aorta sections, suggesting that monosaccharide diffusion is rapid when compared to the rate of fluorescent AGE formation.

Keywords: artery, glycation, autofluorescence, multiphoton imaging

1. INTRODUCTION

Diabetes is a major health problem world-wide. Increased sugar levels lead to tissue glycation, resulting in the formation of Schiff bases which are converted to Amadori products. These intermediate glycation products are eventually converted to long-lived and irreversible advanced glycation end products (AGEs). While tissue glycation occurs with normal aging, AGEs accumulation is accelerated in diabetic patients. AGEs affect normal physiological functions by forming cross-links in connective tissues and by binding to AGEs receptors (RAGEs) which can result in the production of reactive oxygen species.[1-3] AGEs accumulation in tissues result in diabetic complications such as retinopathy, nephropathy, neuropathy, and cardiovascular diseases.[4-7] The roles of AGEs in diabetic pathogenesis also led to concerns as Maillard reaction is accelerated at higher temperatures and leads to food browning.[8, 9] Inhibitors such as aminoguanadine were shown to be effective in reducing diabetic pathogenesis.[6, 10] Other molecules such as 3-phenyacyl-4,5-dimethylthiazolium chloride can improve compliance in stiffened arteries.[11] Among the variety of AGEs, some are fluorescent.[12, 13] Therefore, developing non-invasive diagnostic tool based on the detection of fluorescent AGEs (fAGEs) may be of value in the clinical setting.

Since aorta is a few millimeters in thickness, there should be a gradient of diabetic pathogenesis with a decrease in severity away from the vascular center. Understanding monosaccharide diffusion and the rate of AGEs formation can lead to improved understanding of diabetic pathogenesis. Furthermore, since the rate of glycation is different for different monosaccharides, there is a need to investigate how different sugar molecules affect cardiovascular pathogenesis. The reactivity of monosaccharide with the amino group in forming Schiff's base is found to be in the order of glucose, galactose, and fructose.[14]
In this study, we study the effects of diffusion and fAGEs formation. Disaccharides and polysaccharides such as sucrose, lactose, and starch require enzymatic digestion into monosaccharides before intestinal absorption[15] we will focus on monosaccharides in this study.

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2. MATERIALS AND METHODS

2.1 Preparation of glycated porcine arteries

Porcine aortas was obtained at a local traditional market. Artery sections with length of 20~30 mm were used. Tissue sections were washed in 0.01 M phosphate buffered saline solution (PBS, Sigma-Aldrich, St. Louis, MO, USA), and soaked in 1% povidone-iodine solution for 10 seconds and washed in sterilized PBS solution. Outer surface and two sections of each sterilized aorta were sprayed with polytetrafluoroethylene, then aorta would be soaked in 25 mL glycated solution.

Monosaccharides used were glucose, galactose and fructose. Specifically, we used D-glucose (Sigma-Aldrich, St. Louis, MO), D-galactose (Acros Organics, Fair Lawn, NJ) and D-fructose (Sigma-Aldrich, St. Louis, MO). Glycated solutions were 0.5 M sugar solutions in 0.05 M PBS solution and 1% penicillin-streptomycin (10,000 U/mL, Gibco, CA). Tissue sections were kept in 37 ℃ incubator and the glycated solution was replaced each 4 days. All tissues were incubated under the above conditions for different periods. Tissues were removed from sugar solutions and cut into sections 2.0 mm in thickness for imaging.

2.2 Multiphoton imaging

Multiphoton imaging was performed on a home-built system based on an inverted microscope (TE2000U, Nikon, Japan). 30mW with 780 nm pulse laser was the excitation source of titanium-sapphire (ti-sa) laser (Tsunami®, Spectra Physics, Santa Clara, CA). Fluorescent signal was obtained through focusing objective (20X, S Fluor, 20×/NA 0.75, Nikon, Japan). Single photon counting photomultipliers (PMT, R7400P, Hamamatsu, Hamamatsu City, Japan) were used to detect signal photons.

3. RESULTS AND DISSUSION

The aorta is rich in connective tissues. Elastic fiber is autofluorescent and collagen has second harmonic generation signal due to its non-centrosymmetric structure. In this study, aorta sections are treated with the three monosaccharides of D-glucose, D-galactose and D-fructose. In Figs. 1-3, we show representative images of cross sections of porcine aorta treated with the three monosaccharides. Among the three monosaccharides, the effect of fructose in glycation surpasses that of galactose and glucose.

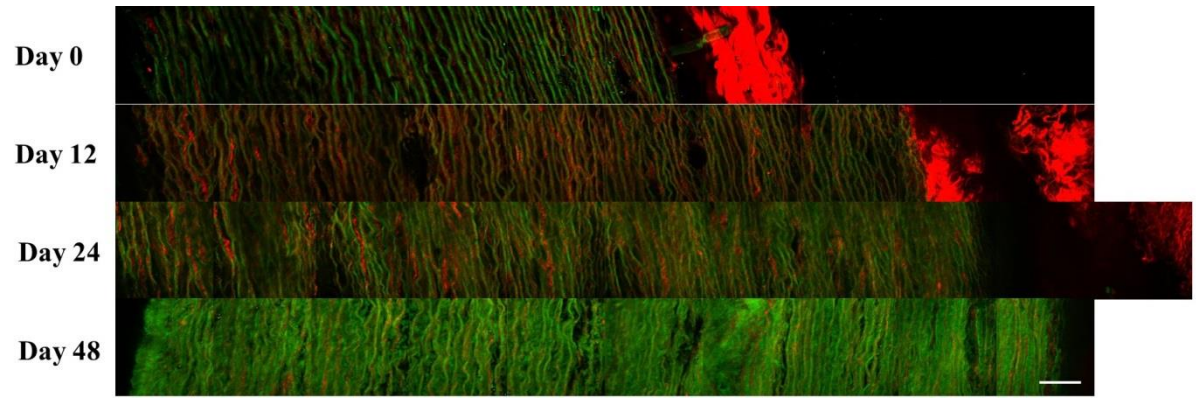

Figure 1. Multiphoton imaging of D-glucose glycated of porcine aorta in Day 0, 12, 24 and 48. Autofluorescence (green) and second harmonic generation (red) signals are presented in aorta. Scale bar is 100 μm

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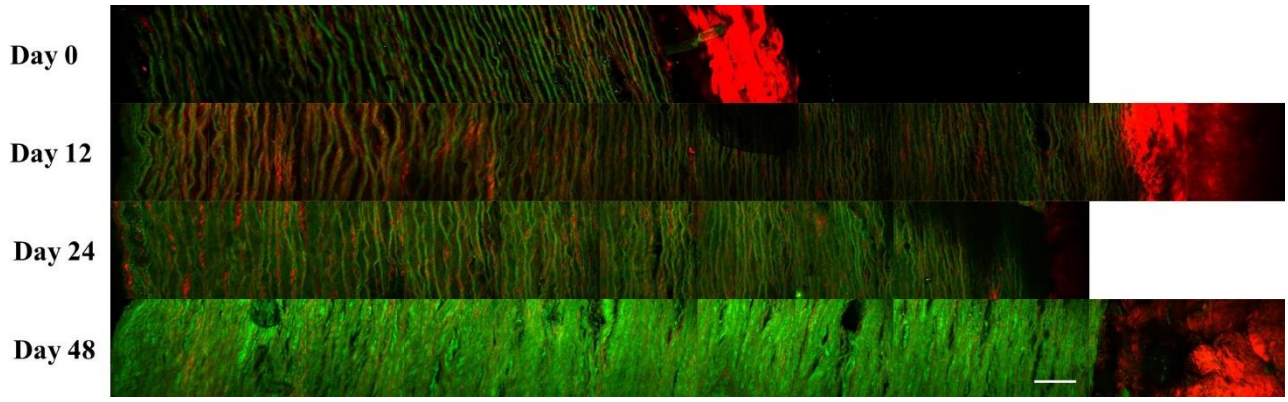

Figure 2. Multiphoton imaging of D-galacotse glycated of porcine aorta in Day 0, 12, 24 and 48. Autofluorescence (green) and second harmonic generation (red) signals are presented in aorta. Scale bar is 100 μm.

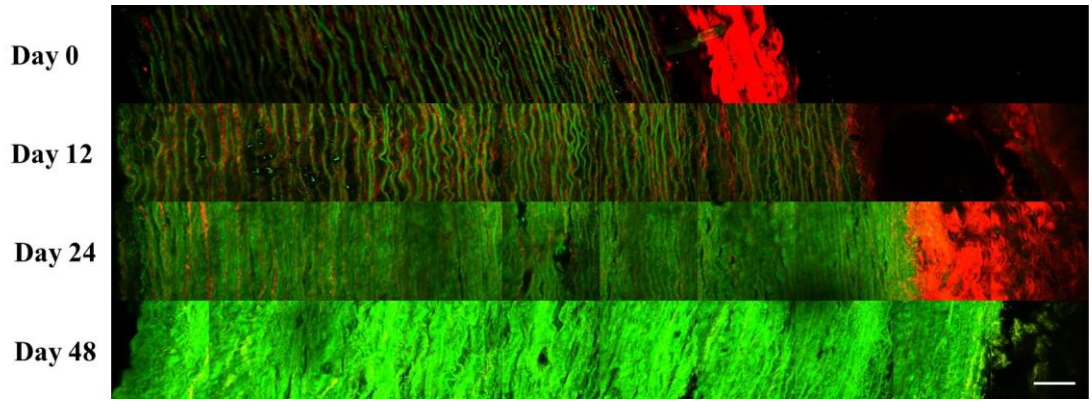

Figure 3. Multiphoton imaging of D-fructose glycated of porcine aorta in Day 0, 24 and 48. Autofluorescence (green) and second harmonic generation (red) signals are presented in aorta. Scale bar is 100 μm.

4. CONCLUSION

In this study, we simulated the effect of monosaccharide induced formation of advanced glycation end products in porcine aorta. After exposing the aorta interior to the three monosaccharides (D-glucose, D-galactose, and D-fructose), we imaged the aorta cross-sections for up to 48 days of incubation. We found that for Days 0 to 48 incubation, autofluorescence is constant along the radial direction of the aorta sections, suggesting that monosaccharide diffusion is rapid in comparison to the rate of formation of fluorescent AGEs (fAGEs). Moreover, we found that in porcine aorta, the rate of fAGE formation of D-fructose is the fastest followed by D-galactose and D-glucose.

5. ACKNOWLEDGEMENTS

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