Multiphoton autofluorescence imaging of advanced glycation end products in glycated tissues

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ABSTRACT

The formation and accumulation of advanced glycation end products (AGEs) contribute to diabetic complications such as retinopathy, neuropathy, nephropathy, and cardiovascular diseases. It is clear that the development of effective technique in AGEs detection 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. Since some AGE are fluorescent, we propose to investigate the degree of tissue glycation in forming fluorescent AGEs (fAGEs). In this preliminary study, we will investigate the effects of glucose, fructose, and galactose, three of the most abundant dietary simple sugars, in fAGEs production. Excised tissues will be treated in solutions containing the three sugar types; multiphoton autofluorescence imaging will then be performed on the treated tissues to determine their autofluorescence levels.

Keywords: advanced glycation end products (AGEs), autofluorescence, multiphoton imaging

1. INTRODUCTION

In recent years, humans have consumed increasing more carbohydrate [1]. Over consumption of sugars is a major contributing factor of diabetes. In diabetic patients, prolonged exposure to elevated sugar molecules would lead to tissue damage. Elevated sugar levels lead to the formation of advanced glycation end products (AGEs) through Maillard reaction [2, 3]. Some AGEs, such as, crossline, fluorolink, and pentosidine, are fluorescent. Tissue glycation occurs in different tissues including that in collagen. Since collagen is the most abundant mammalian protein, it may act as a target tissues form which to detect fluorescent AGEs (fAGEs). In our group, we have previously studied the effect of ribose in formation fAGEs [4]. In this study, we expand upon the previous study in studying the effect of commonly consumed sugars in forming fAGEs.

2. MATERIALS AND METHODS

2.1 Preparation of glycated tissues

Porcine tissues were obtained from a local market. We used five samples including cornea, tendon, aorta, kidney and skin. Corneas was obtained from the porcine eye, with the epithelium scraped from cornea surface. Furthermore, corneas were punched through the use of a 8.0 mm tissue punch (Harris Uni-Core, PA, USA), and the thickness of porcine cornea is about 2–3 mm. Tendon were obtained from the knee, with each specimen about 10 × 5 × 2 mm$^3$ in size. Aorta tissue with inner diameter in the range of 8–10 mm and outer diameter in the range of 10–15 mm. The aorta tissue was

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cut into as about 2 mm length sections. Kidney would be cut into pieces along the cross-section, each kidney tissue was obtained about 5 × 5 × 2 mm³ from the renal cortex. Skin tissue would be obtained from porcine back skin, each approximately 5 × 5 × 2 mm³ in volume. All tissues 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.

All tissues were glycated in three types of 0.5 M sugar solutions. The tissues were kept at 37°C and 5% CO₂. The control incubation condition was 5 mL of 0.05 M PBS and 1% penicillin-streptomycin (Gibco, CA). These three types of sugars used were glucose (Sigma-Aldrich, St. Louis, MO), galactose (Sigma-Aldrich, St. Louis, MO) and fructose (Sigma-Aldrich, St. Louis, MO). All tissues were incubated under the above conditions for different periods of 6, 12, 18, 24 and 30 days.

2.2 Multiphoton autofluorescence imaging

All samples were covered with a 0.17 mm thickness cover glass for multiphoton microscopy imaging. Autofluorescence signal was obtained via multiphoton fluorescence microscopy, second harmonic generation (SHG) was also obtained. The autofluorescence detection bandwidth was from 435 to 630 nm, and SHG was detected from 380 to 400 nm. All autofluorescence image was rendered with green, SHG was rendered with red.

2.3 Data analysis

GNU Octave software was used to separate out the pixel with collagen and without collagen. ImageJ was used to merge autofluorescence and SHG images. OriginPro® was used for analyzing autofluorescence data.

3. RESULTS AND DISCUSSION

Shown in Figure 1 are the multiphoton images of cornea and tendon at different time points. In these tissues, collagen was the main tissue component. Increase in autofluorescence is attributed to increase in fAGEs. With increased incubation period, there is a marked increase in tissue autofluorescence.

Figure 1. Autofluorescence (green) and second harmonic generation (red) images of glycated A). cornea and B). tendon. The tissues were treated in the three different sugar types.
Figure 2. Autofluorescence (green) and second harmonic generation (red) images of glycated A). aorta and B). dermis. The tissues were treated in the three different sugar types.

In addition to imaging, we also analyzed the temporal profiles of tissue autofluorescence from the different sugar molecules. Shown in Fig. 3 are the autofluorescence signals of different tissue types up to 30 days.
4. CONCLUSION

The formation and accumulation of advanced glycation end products (AGE) contribute to diabetic complications such as retinopathy, neuropathy, nephropathy, and cardiovascular diseases. It is clear that the development of effective technique in AGE detection 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. Since some AGE are fluorescent, therefore, we propose to investigate the use of fluorescent AGE (fAGE) in selected tissues to predict the degree of tissue pathogenesis. Specifically, we studied the effects of glucose, fructose, and galactose in the production of fAGE in different tissues components. The excised tissues were treated in solutions containing the different sugars. Multiphoton imaging was then performed on the treated tissues to determine the tissues’ autofluorescence properties. We found that with increased time, tissue autofluorescence tends to increase, indicative of further accumulation of fAGEs in the tissue components. The result of this study may lead to the development of non-invasive fAGE detection technology in pathogenic diagnosis in diabetic patients.

Figure 3. Temporal autofluorescence profiles of A) cornea, B) tendon, C) collagen of aorta, D) elastic fiber of aorta, E) collagen of dermis, and F) elastic fiber of dermis.
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