Vascular Lesions in Diabetic Retinopathy

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Abstract

Diabetic retinopathy is the leading cause of blindness in the working-age population. Two prominent vascular lesions that occur early in diabetic retinopathy are acellular capillaries and pericyte ghosts. These retinal vascular lesions develop due to the loss of endothelial cells and pericytes in the diabetic retina. This study seeks to compare the number of these vascular lesions in retinas affected by the hyperglycemic conditions of diabetes and in wild-type retinas. In order to visualize the capillary networks of retinas and search for lesions, one must use a novel technique known as retinal trypsin digest (RTD). Scientists investigating diabetic retinopathy use RTD to isolate the intact retinal capillary network by first isolating the intact retina from the rest of the eye, then digesting the retina in trypsin, then carefully removing excess glial tissue, and finally mounting the vasculature on a silane-coated slide. The resulting capillary network is then stained with periodic acid-Schiff (PAS) and hematoxylin. PAS stains the basement membrane pink and hematoxylin stains the nuclei of cells bluish-purple; these stains make lesions much more apparent under a microscope. At the end of this experiment, the images of the RTD showed that there are more lesions in diabetic retinas than normal retinas. RTD and subsequent staining expose the damage done to the retinal vasculature by hyperglycemia and diabetes mellitus. This technique is indispensible to the study of acellular capillaries and pericyte ghosts and is a valuable tool for the overall understanding of diabetic retinopathy.

Introduction

Diabetes mellitus is one of the most prevalent endocrine diseases in modern society, causing excess blood glucose due to disturbances in the production and effectiveness of insulin. The hormone insulin normally helps decrease blood glucose levels by signaling cells in the body to absorb glucose from the blood; unfortunately, this process is interrupted in type 1 and type 2 diabetes. In type 1 diabetes, which affects 5% to 10% of those with diabetes mellitus, the beta cells of the pancreas have a genetic mutation that prevents the production of functioning insulin. In type 2 diabetes, which affects 90% to 95% of those with diabetes mellitus, the cells of the body develop a resistance to insulin, either due to reduced sensitivity of insulin receptors or reduced numbers of insulin receptors. Both types of diabetes mellitus, however, result in hyperglycemic conditions in the blood.

These hyperglycemic conditions result in a variety of complications throughout the body, including nephropathy and neuropathy. In particular, diabetes-induced hyperglycemia causes a retinal disease known as diabetic retinopathy[2]. Diabetic retinopathy causes major damage to the retinal vasculature and can eventually result in retinal detachment and blindness.

Hyperglycemia causes the abnormal regulation of various genes in pericytes—contractile cells that control the flow of blood through retinal capillaries—and endothelial cells that line the walls of the capillaries. This damage eventually results in apoptosis and the loss of endothelial cells and pericytes. Lost pericytes leave behind a cavity in the capillary wall known as a pericyte ghost and lost endothelial cells leave behind acellular capillaries that can no longer function properly. Pericyte ghosts and acellular capillaries are the two most prominent lesions in the early stages of diabetic retinopathy and indicate future damage to the retina. These vascular lesions are indicative of how advanced diabetic retinopathy is, and so their numbers can be used as raw data for comparison of the damage to different retinas.

In order to count the retinal vascular lesions, one must use a technique called retina trypsin digest (RTD) to isolate the intact retinal capillary network. Developed by David Cogan and Toichiro Kuwabara in 1960[1], this novel technique isolates the retinal capillary network from the surrounding glial tissue, allowing for thorough analysis of the vasculature's finer details. In particular, RTD allows for the counting of the vascular lesions present in retinas and is therefore vitally important to the study of diabetic retinopathy.

Methods

Isolating the Intact Retina:

1. Mouse eye soaked in formalin for at least 48 hours after enucleation
2. Anterior part cut off by razor blade along ora serrata
3. Lens removed from posterior eye with microtweezers
4. Retina separated from surrounding sclera and choroid
5. Incubate retina in glycine buffer for 24-48 hours

Digestion in Trypsin:

1. Incubate retina in 3% trypsin buffer in 37°C water bath for 20 minutes
2. Repeat three more times, replacing trypsin each time

Cross-Hatching and Mounting:

1. Internal limiting membrane separated from rest of retina
2. Pat down retina using brushes to remove excess glial tissue
3. Vasculature mounted on silane-coated slide after fully cleaned

Staining:

1. Periodic acid-Schiff (PAS) stains basement membrane pink
2. Hematoxylin stains nuclei bluish-purple

Results

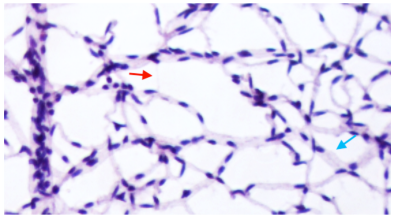

Figure 1. A picture of the isolated, intact retinal capillary network from a wild-type mouse (200x magnification). The purple dots are the nuclei of endothelial cells and pericytes. Red arrows point to acellular capillaries and blue arrows point to pericyte ghosts.

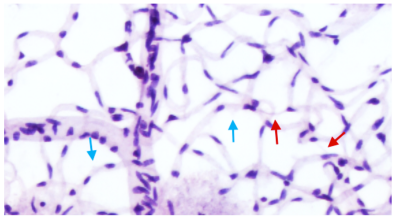

Figure 2. A picture of the isolated, intact retinal capillary network from a diabetic mouse (200x magnification). The purple dots are the nuclei of endothelial cells and pericytes. The blurred regions are areas where excess glia has not been cleaned out completely. Red arrows point to acellular capillaries and blue arrows point to pericyte ghosts.

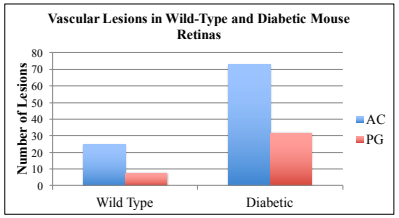

Figure 3. A graph comparing the numbers of acellular capillaries (AC) and pericyte ghosts (PG) in wild-type and diabetic retinas. Two retinas were analyzed for each group. The graph represents the total combined number of lesions.

Summary

As demonstrated by the data, many more vascular lesions occurred in the diabetic mouse retina than in the wild-type mouse retina. Specifically, the wild-type retina had 25 acellular capillaries and 8 pericyte ghosts, for a total of 33 vascular lesions. The diabetic retina, on the other hand, had 73 acellular capillaries and 32 pericyte ghosts, for a total of 105 vascular lesions.

The excess vascular lesions in the diabetic mouse retina demonstrate the damage done to the retina by the hyperglycemic conditions of diabetes mellitus. High glucose alters the expression of various important genes and may cause apoptosis of endothelial cells and pericytes.

Diabetic mouse retinas are reliable models for the pathology of diabetic retinopathy in humans since both species experience similar vascular lesions in response to hyperglycemia.

Retinal trypsin digest (RTD) proves to be a reliable tool for the study of retinal vasculature, and more specifically, retinal vascular lesions. The periodic acid-Schiff (PAS) and hematoxylin stains also proved reliable at making vascular lesions visible under the microscope.

Conclusions

The hyperglycemic conditions of diabetes mellitus caused structural damage to the retinal vascular network. In particular, hyperglycemia disrupted normal expression of key genes, resulting in apoptosis of endothelial cells and pericytes lining the capillary walls. This cell death resulted in lesions such as acellular capillaries and pericyte ghosts. Therefore, these lesions occur in greater numbers in diabetic retinas than in normal retinas.

RTD and subsequent staining with periodic acid-Schiff's reagent and hematoxylin enabled the visualization of such structural damage and hence a count of the relative numbers of lesions in the retinas. This procedure enabled a reliable visualization of the damage caused by diabetes-induced hyperglycemia.

Visualizing the retinal damage caused by hyperglycemic conditions allows for future research to focus on understanding the underlying pathologies that cause diabetic retinopathy and its possible impact on clarity of vision.

References

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2. 2. ENGERMEN RL. Pathogenesis of Diabetic Retinopathy. *Diabetes.* 1989 Oct; 38(10): 1203-1206.

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