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Hyperlipidemia and the development of diabetic retinopathy: Comparison between type 1 and type 2 animal models

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ABSTRACT

Aim. In the pathogenesis of diabetic retinopathy, reactive oxygen species (ROS) are elevated in the retina and the mitochondria are damaged, resulting in accelerated apoptosis. Dyslipidemia is also considered as one of the major factors in its development, and our aim is to investigate the compounding effect of hyperlipidemia in retinopathy.

Methods. Retinal ROS, mitochondrial damage and vascular pathology were investigated in Zucker diabetic fatty rats (ZDF, type 2 diabetes model), during the age that spans from hyperlipidemia/pre-hyperglycemia (6 weeks), to severe hyperglycemia/moderate hyperlipidemia (~12 weeks), and ultimately to severe hyperglycemia/hyperlipidemia (20–40 weeks). For comparison, retina from streptozotocin-induced Wistar rats (type 1 diabetic for 10–40 weeks) was analyzed.

Results. Compared to age-matched lean rats, despite increased retinal cytosolic ROS in 6-week-old ZDF rats, mitochondrial dysfunction and DNA damage were not detected, and in 12-week-old ZDF rats, retinal mitochondria were dysfunctional, but mtDNA damage and vascular pathology (cell apoptosis and degenerative capillaries) were not detectable. Retina from 20-week-old ZDF rats (hyperglycemic for 14 weeks or less) had significant mitochondrial dysfunction, mtDNA damage and vascular pathology, and similar abnormalities were observed in 40-week-old ZDF rats. Although retinal mitochondrial dysfunction was observed in Wistar rats diabetic for 20 weeks, mtDNA damage and vascular pathology were not detectable till the duration of diabetes was further extended.

Conclusions. Hyperlipidemia, in a hyperglycemic milieu, potentiates mitochondrial damage and augments the development of retinopathy. Control of dyslipidemia in pre-diabetic patients may prevent/delay the development and the progression of this devastating disease.

Abbreviations: DAPI, 4″,6-diamidino-2-phenylindole, dihydrochloride; DCHFDA, 2′,7′-dichlorofluorescein diacetate; ERG, electroretinogram; ETDRS, Early Treatment of Diabetic Retinopathy Study; FITC, fluorescein isothiocyanate; GHb, glycated hemoglobin; mtDNA, mitochondrial DNA; LN, lean; ND, not determined; Nox2, NADPH oxidase 2; Rac1, Ras-related C3 botulinum toxin substrate 1; ROS, reactive oxygen species; TUNEL, terminal deoxyribonucleotide transferase-mediated dUTP nick-end label; VLDL, very low-density lipoprotein; Wks, weeks; ZDF, Zucker diabetic fatty.

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1. Introduction

Diabetes has become now the global epidemic of 21st century, and by 2031, 600 million people are projected to suffer from this disease. Among those, 90%–95% cases will have type 2 diabetes, and obesity is considered as one of the major contributors to these staggering numbers. Type 2 diabetes comes with an additional burden of moderate to severe hyperlipidemia, and the characteristic features of diabetic dyslipidemia are high plasma triglycerides and low-density lipoprotein cholesterol (LDL) and reduced levels of high density lipoprotein cholesterol. These patients are at substantially increased risk for developing macrovascular and microvascular complications including retinopathy and nephropathy [1,2]. Retinopathy, a slow progressing disease, affects vast majority of patients with 20–25 years of diabetes. The pathogenesis of this blinding disease is complex and many biochemical, metabolic and molecular mechanisms are implicated in its development [3,4]. Our previous work has shown that during initial stages of the disease, NADPH oxidase-2 (Nox2) is activated producing reactive oxygen species (ROS), and these ROS damage the mitochondria to initiate the apoptotic machinery, a phenomenon, which precedes the development of histopathology characteristic of diabetic retinopathy. Damaged mitochondrial DNA (mtDNA) compromises the electron transport system and continues to fuel into a futile cycle of free radicals [4–10]. Although hyperglycemia remains as one of the major instigators of the development of diabetic retinopathy, other systemic factors, including hyperlipidemia and hypertension are also closely associated with its development [11–13]. The Early Treatment of Diabetic Retinopathy Study (ETDRS) has shown a relationship between retinal hard exudates and total and LDL cholesterol [14]. In addition, clinical trials using long-term administration of lipid-lowering therapy, fenofibrate, has documented a significant relative reduction of progression of diabetic retinopathy in patients with background retinopathy [15]. In a rat model of type 2 diabetes, dietary supplementation with docosahexaenoic acid prevents the development of retinopathy [16]. In vitro models have also shown a close correlation between lipoxygenase pathway and vascular permeability, and between modified LDL and pericyte loss in diabetic retinopathy [17–19]. Recent study from our laboratory using retinal endothelial cells has further documented the role of lipotoxicity, and the results have shown that in a glucotoxic environment, the addition of lipotoxicity exacerbates ROS production and mtDNA damage [10]. The exact mechanism by which hyperlipidemia impacts the development of retinopathy in type 2 diabetic patients, however, remains elusive.

The aim of this study is to investigate how hyperlipidemia contributes to the development of diabetic retinopathy. Mitochondrial damage and vascular pathology were investigated in the retina from Zucker diabetic fatty (ZDF) rats during the age that spans from pre-diabetic, but hyperlipidemic (6 weeks), to overt hyperglycemic and hyperlipidemic (40 weeks). For comparison, the retina from streptozotocin-induced Wistar rats (type 1 diabetes) was also analyzed at duration of diabetes when retinal mitochondria are intact and the histopathology characteristic of diabetic retinopathy is not detectable (10 weeks of diabetes), to the duration when mitochondrial damage and histopathology can be detected in the retinal vasculature (40 weeks of diabetes).

2. Methods

2.1. Animal Models

Male, ZDF rats (Charles River Laboratories, Wilmington, MA) at 6 weeks (normal glycemia with mild hyperlipidemia), 12 weeks (hyperglycemia and moderate hyperlipidemia), and 20–40 weeks of age (hyperglycemia and overt hyperlipidemia) were used as type 2 diabetes animal model [20]. For comparison with type 1 diabetic animal model, diabetes was induced in 7–8-week-old male Wistar rats (Harlan Laboratories, Indianapolis, IN) by a single injection of streptozotocin (55 mg/kg BW), and the rats were maintained diabetic for 10–40 weeks. Age-matched Zucker lean rats and normal Wistar rats served as their respective controls. The entire animal colony was weighed two times/week, non-fasting blood glucose was monitored once every week, and glycated hemoglobin (GHb) was quantified every 8–9 weeks. While ZDF rats started to present hyperglycemia around 7 weeks of age, Wistar rats were hyperglycemic within 3 days of streptozotocin administration. Animals were sacrificed by CO_2 asphyxiation, and blood (2–3 mL) was collected by heart puncture. One eye was fixed in formalin for histopathology, and the retina isolated from the other eye was frozen immediately in liquid nitrogen, as reported previously by us [6,9,21]. Treatment of the animals conformed to the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research and NIH guidelines, and was approved by the Wayne State University's Animal Care and Use Committee.

2.1.1. Serum Lipid Profile

Serum triglycerides and cholesterol/triglyceride-rich very low-density lipoprotein (VLDL) were quantified using commercial assay kits (#ab65336 and ab65390 respectively from Abcam, Cambridge, MA) following the manufacturer's instructions.

2.2. Retinal Damage

2.2.1. Mitochondrial dysfunction and damage were investigated by evaluating their membrane permeability, activity of the electron transport system and mtDNA damage

Mitochondria were isolated from the retina using mitochondria isolation kit and following the manufacturer's instructions (Pierce, Rockford, IL); these preparations are devoid of nuclear contamination [21]. Permeability of the mitochondrial membrane was quantified by measuring its swelling; briefly, mitochondrial protein (5–10 μg) was equilibrated for 30 s at 25 °C in an a 60 μL assay volume containing 3 mmol/L HEPES buffer (pH 7.4) supplemented with 215 mmol/L mannitol, 71 mmol/L sucrose and 5 mmol/L succinate. To induce the transition, calcium chloride (400 μmol/L) was added, and a decrease in the absorbance at 540 nm was followed for 5 min.