CHARACTERIZATION OF EARLY RETINAL DEGENERATION IN ZDF RATS, A GENETIC MODEL OF TYPE 2 DIABETES

Fernandez-Bueno I.1,2, Jones R3, Lopez-García A3, Cheetham S3, Diebold Y1

1Instituto Universitario de Oftalmobiologia Aplicada (IOBA), University of Valladolid, Valladolid, Spain. 2 Centro en Red de Medicina Regenerativa y Terapia Celular, Castille & Leon Regional Government Action, Spain. 3RenaSci Limited, Nottingham, UK

INTRODUCTION

Diabetic retinopathy (DR) is a microvascular complication associated with chronic exposure to hyperglycemia and is a major cause of blindness worldwide.1 Although clinical assessment and retinal autopy of diabetic patients provide information on the features and progression of DR, its underlying pathophysiological mechanism cannot be deduced.2 In order to have a better understanding of the development of DR at the molecular and cellular levels, and to evaluate the effect of candidate drugs properly, reliable and appropriate animal models are required.3 Zucker Diabetic Fatty (ZDF) rats are a genetic model of type 2 diabetes. They carry an inherited obesity gene mutation, which results in impairment of glucose tolerance and insulin resistance. Excessive body weight gain was observed in male ZDF rats in the first 6 months of life, but the weight decreases to a level similar to the lean controls afterwards.4 Hyperglycemia starts at 6 to 7 weeks of age and maintains high throughout their life.5 Retinopathological studies in these rats have mainly focused on the vasculature. Thickening of the capillary basement membrane, increased capillary cell nuclear density, apoptosis of endothelial cells and pericytes, increased number of asecular capillaries and pericyte ghostes, were reported after several months of hyperglycemia in these rats compared to the corresponding lean controls.2,6 However, retinal functional analysis and morphological studies of the retinal neuronal and glial cells in the ZDF rats remain to be elucidated.

METHODS

- Male Lean (?/+; n=6) and Obese ZDF (fa/fa; n=6) rats (Charles River, UK) were used and maintained on a Purina 2008 diet
- At 24 weeks of age and freely feeding blood glucose levels and body weights were determined and animals euthanized
- Eyes were embedded in paraffin wax
- Ocular serial sections were stained for hematoxylin and eosin (HE) and immunostained for Tunel and/or Hoescht
- Phenotype-specific markers: recoverin, vimentin, glial fibrillary acidic protein (GFAP), retinal pigment epithelium-specific 65 kDa protein (RPE65) and zonula occludens protein 1 (ZO1)

RESULTS I

Body weights were not significantly different between Lean (4016±4.4g) and Obese (391±4.7g) animals. Blood glucose levels were significantly higher in Obese rats compared to Lean controls (30.8±1.0mM and 10.6±0.6mM, respectively)

RESULTS II

Fig 1. HE staining of Lean (A) and Obese (B) ZDF ocular sections. Retinal layers were easily recognized through the retinal parenchyma. In Obese rats subtotal ONL and INL misorganization and photoreceptor nuclei outside the OLM were observed (B, arrowheads). TUNEL labeling in Lean (C) and Obese (D) ZDF rat retina. TUNEL-positive elements were detectable only occasionally, however, only Obese animals show TUNEL-positive nuclei located in the photoreceptor IS area (D, arrowheads). Recoverin (Rec) expression in Lean (E) and Obese (F) ZDF rats. Rec stains photoreceptors of the ONL and cone bipolar cells in the INL. Slightly reduction in Rec staining intensity was observed in Obese ZDF animals. Hoescht: blue. Ch: choroid; GCL: ganglion cell layer; INL: inner nuclear layer; ONL: outer nuclear layer; RPE: retinal pigment epithelium. * and ** artifacts due to processing. Scale bars: 25m (A-D) and 20m (E&F)

Fig 2. Vimentin (Vim) and GFAP expression in Lean (A-C) and Obese (D-F) retinal sections of ZDF rats. In Lean animals Vim stains Müller cells cytoplasm to the outer plexiform layer and GFAP was limited to the innermost layers of the neuroretina. In Obese ZDF, Vim labeling extended to the ONL with thickened lateral branches at the OPL (D, arrowheads) and GFAP was partially regulated throughout the cytoplasm of the Müller cells to the ONL. Vimentin often co-localized in Müller cells with GFAP (yellow; C&F). RPE65 and ZO1 expression in Lean (G-I) and Obese (J-L) RPE-choroid of ZDF rats. RPE65 stains the soma of RPE cells with no apical processes labeling. ZO1 was weakly detected in the borders of the RPE cells, ZO1 was distributed throughout the ONL to the RPE nuclei in Lean and appeared diffuse in Obese rats (H&K, arrowheads). Hoescht: blue. Ch: choroid; INL: inner nuclear layer; ONL: outer nuclear layer; RPE: retinal pigment epithelium. Scale bars: 25m (A-F) and 20m (G-L)

CONCLUSIONS

To morphologically characterize early retinal degeneration in Obese Zucker Diabetic Fatty (ZDF) rats, a genetic model of type 2 diabetes, by histological and immuno-histochemical studies


Support:
EU Program FP7-PEOPLE-2013-IAPP (61211/3D-Net). I. Fernandez-Bueno was supported by Centro en Red de Medicina Regenerativa y Terapia Celular, Castille & Leon Regional Government Action, Spain
Financial Disclosures:
Fernandez-Bueno I, none; Jones R, none; Lopez-Garcia A, none; Cheetham S, none; Diebold Y, none

References: