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Research report

Lack of Galectin-3 attenuates neuroinflammation and protects the retina and optic nerve of diabetic mice

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ABSTRACT

Diabetic retinopathy is the leading cause of acquired blindness in working-age individuals. Recent work has revealed that neurodegeneration occurs earlier than vascular insult and that distal optic nerve damage precedes retinal degeneration and vascular insult. Since we have shown that optic nerve degeneration is reduced after optic nerve crush in Galectin-3 knockout (Gal-3 −/−) mice, we decided to investigate whether Gal-3 −/− could relieve inflammation and preserve both neurons and the structure of the retina and optic nerve following 8 weeks of diabetes. Diabetes was induced in 2-month-old male C57/bl6 WT or Gal-3 −/− mice by a single injection of streptozotocin (160 mg/kg). Histomorphometric retinal analyses showed no gross difference, except for a reduced number of retinal ganglion cells in WT diabetic mice, correlated to increased apoptosis. In the optic nerve, Gal-3 −/− mice showed reduced neuroinflammation, suggested by the smaller number of Iba1+ cells, particularly the amoeboid profiles in the distal end. Furthermore, iNOS staining was reduced in the optic nerves of Gal-3 −/− mice, as well as GFAP in the distal segment of the optic nerve. Finally, optic nerve histomorphometric analyses revealed that the number of myelinated fibers was higher in the Gal-3 −/− mice and myelin was more rectilinear compared to WT diabetic mice. Therefore, the present study provided evidence that Gal-3 is a central target that stimulates neuroinflammation and impairs neurological outcomes in visual complications of diabetes. Our findings provide support for the clinical use of Gal-3 inhibitors against diabetic visual complications in the near future.

1. Introduction

Diabetes is currently one of the main chronic world pandemics, estimated to affect 415 million people worldwide. Diabetic retinopathy is considered the most common cause of acquired visual defects, being responsible for 1.9% of all cases of severe visual impairment and 2.6% of all cases of blindness (International Diabetes Federation (IDF), 2015). Before the vascular changes, the clinical characteristic used to diagnose diabetic retinopathy, diabetes increases the number of immune cells (Krady et al., 2005), produces reactive oxygen species (Carpi-Santos et al., 2016) and causes ganglion cell death in the retina (Martin et al., 2004), associated with activation of macrophage/microglia and astrocytes in the optic nerve, leading to disorganized myelination and reduction in the number of nerve fibers (Fernandez et al., 2012).

Neuroinflammation causes proliferation of glial cells and glial scar formation, synaptic impairment, and neuronal and glial cell death, contributing to neurological and neurodegenerative diseases (Cerami et al., 2017). Among its major players, Galectin-3 (Gal-3) is a galactoside-binding lectin composed of one or two carbohydrate-recognition domains, and plays an important role as an immunomodulatory mediator, exacerbating or reducing neuroinflammation, depending on each particular case (Shin, 2013).

Our group demonstrated the dual role of Gal-3 in neuroinflammation of the peripheral versus central nervous system after

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trauma. We found that Gal-3 −/− mice showed higher phagocytic activity of macrophages and Schwann cells, combined with increased secretion of pro-inflammatory cytokines, which was accompanied by more-rapid Wallerian degeneration, after a traumatic sciatic nerve lesion (Narciso et al., 2009; Mietto et al., 2013). On the other hand, after spinal-cord or optic nerve lesion, Gal-3 −/− mice showed fewer microglia/macrophages in the lesion, preferentially polarized to an anti-inflammatory profile, resulting in spared white matter (Mostacada et al., 2015) and reduced retinal ganglion-cell apoptosis (Abreu et al., 2017). Interestingly, Gal-3 is upregulated in diabetic retinopathy and might be implicated in the vascular pathology of diabetes (Canning et al., 2007).

Since our previous reports showed that the absence of Gal-3 was able to protect CNS tissue from traumatic lesion, in the present study we investigated whether the absence of Gal-3 could relieve the neuroinflammatory and morphological features of diabetes in the retina and optic nerve.

2. Results

2.1. Blood glucose levels and body weight

One and 8 weeks after diabetes induction, non-fasting blood-glucose levels were higher than 350 mg/dL in both WT and Gal-3 −/− diabetic animals. No animals from either group had glucose levels equal to or higher than 350 mg/dL prior to diabetes induction. Besides, body weight in WT animals were significantly lower after 8 weeks of diabetes, while no weight loss was detected in Gal-3 −/− mice (Table 1).

2.2. Lack of Gal-3 protects RGC from apoptosis in diabetes

Since diabetes is known to cause several degenerating events within the retina (Wong et al., 2016), we evaluated whether Gal-3 absence was able to protect the retinal structure, as evaluated in toluidine blue-stained semithin sections (Fig. 1A, C).

Table 1
Follow-up of glycemia and body weight of diabetes-induced animals.

	Glycemia (mg/dL)			Body weight (g)		
	Before induction	1 week after induction	8 weeks after induction	Before induction	1 week after induction	8 weeks after induction
Diabetic WT	170.42 ± 9.36	434 ± 35.54	467.71 ± 73.89	21.84 ± 1.87	21.52 ± 2.071	17.39 ± 3.67**
Diabetic Gal-3 −/−	143.28 ± 11.84	367.57 ± 15.42	395.28 ± 27.56	23.6 ± 1.37	21.93 ± 1.95	21.41 ± 2.01

** Represents $p < 0.01$. n = 7 for each group.

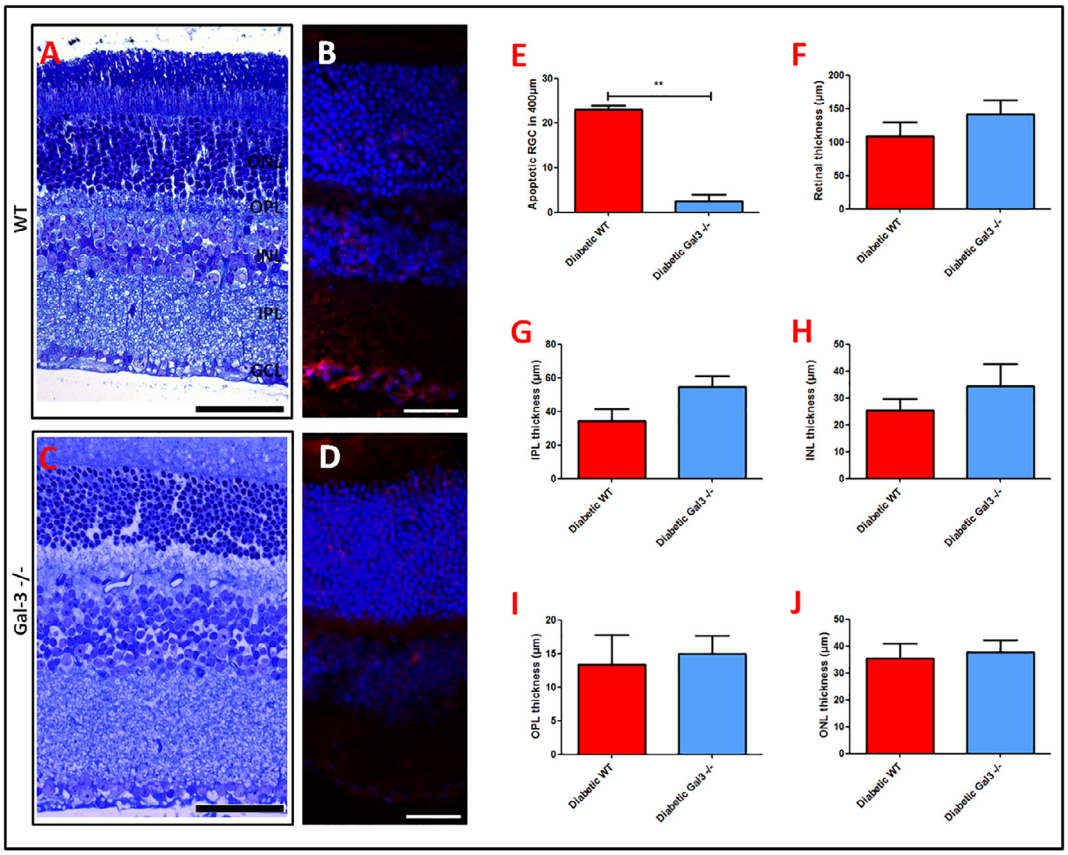

Fig. 1. Absence of Galectin-3 protects against RGC apoptosis in diabetic mice. Representative photomicrographs showing the histologic appearance of toluidine blue-stained retinal sections from diabetic WT (A) and Gal-3−/− (C) mice. Graphs represent data from morphometric analyses, with no changes in the thickness of the retina (F), IPL (G), INL (H), OPL (I) and ONL (J). Representative photomicrographs showing TUNEL staining of retinal sections from diabetic WT (B) and Gal-3−/− (D) mice. Graph showed that absence of Gal-3 reduced the number of TUNEL + cells (E). ** Represents $p < 0.01$. Scale bar: (A–C) 50 µm; (B-D) 25 µm. n = 3 for each group of toluidine blue and TUNEL analysis.