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Time-dependent retinal ganglion cell loss, microglial activation and blood-retina-barrier tightness in an acute model of ocular hypertension

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ABSTRACT

Glaucoma is a group of neurodegenerative diseases characterized by the progressive loss of retinal ganglion cells (RGCs) and their axons, and is the second leading cause of blindness worldwide. Elevated intraocular pressure is a well known risk factor for the development of glaucomatous optic neuropathy and pharmacological or surgical lowering of intraocular pressure represents a standard procedure in glaucoma treatment. However, the treatment options are limited and although lowering of intraocular pressure impedes disease progression, glaucoma cannot be cured by the currently available therapy concepts. In an acute short-term ocular hypertension model in rat, we characterize RGC loss, but also microglial cell activation and vascular alterations of the retina at certain time points. The combination of these three parameters might facilitate a better evaluation of the disease progression, and could further serve as a new model to test novel treatment strategies at certain time points. Acute ocular hypertension (OHT) was induced by the injection of magnetic microbeads into the rat anterior chamber angle ($n = 22$) with magnetic position control, leading to constant elevation of IOP. At certain time points post injection (4d, 7d, 10d, 14d and 21d), RGC loss, microglial activation, and microvascular pericyte (PC) coverage was analyzed using immunohistochemistry with corresponding specific markers (Brn3a, Iba1, NG2). Additionally, the tightness of the retinal vasculature was determined via injections of Texas Red labeled dextran (10 kDa) and subsequently analyzed for vascular leakage. For documentation, confocal laser-scanning microscopy was used, followed by cell counts, capillary length measurements and morphological and statistical analysis. The injection of magnetic microbeads led to a progressive loss of RGCs at the five time points investigated (20.07%, 29.52%, 41.80%, 61.40% and 76.57%). Microglial cells increased in number and displayed an activated morphology, as revealed by Iba1-positive cell number (150.23%, 175%, 429.25%,486.72% and 544.78%) and particle size analysis (205.49%, 203.37%, 412.84%, 333.37% and 299.77%) compared to contralateral control eyes. Pericyte coverage (NG2-positive PC/mm) displayed a significant reduction after 7d of OHT in central, and after 7d and 10d in peripheral retina. Despite these alterations, the tightness of the retinal vasculature remained unaltered at 14 and 21 days after OHT induction. While vascular tightness was unchanged in the course of OHT, a progressive loss of RGCs and activation of microglial cells was detected. Since a significant loss in RGCs was observed already at day 4

Abbreviations: PC, pericytes; BRB, blood retina barrier; RGC, retinal ganglion cells; IOP, intraocular pressure; OHT, ocular hypertension; i.p., intra peritoneal; i.v., intra venous.

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of experimental glaucoma, and since activated microglia peaked at day 10, we determined a time frame of 7–14 days after MB injection as potential optimum to study glaucoma mechanisms in this model.

1. Introduction

In the western industrialized countries glaucoma is one of the most prevalent eye diseases leading to visual impairment and blindness. This disease is characterized by a progressive loss of retinal ganglion cells (RGCs) and their axons (optic nerve fibers), with concomitant loss of the visual field. Currently, this disease is recognized as a multifactorial, progressive neurodegenerative disorder. The exact pathogenesis of glaucoma is not clear, however, one of the major risk factors is elevated intraocular pressure (IOP) (reviewed in (Tamm et al., 2013a, 2013b)). The most common strategy to slow down glaucoma progression is a reduction in IOP by pharmacological or surgical intervention. However, despite this therapeutic approach, many patients show a progression in visual field loss and RGCs death. This indicates that either IOP is not the only cause for glaucoma or that IOP triggers processes, which are continuing despite IOP reduction. Further, also 30–50% of all patients with open angle glaucoma develop optic nerve degeneration without signs of elevated IOP (Anderson et al., 2003). Considering the shortcomings of available treatment options, and with regard to the still incomplete understanding of the pathophysiological processes of glaucoma, new strategies are urgently needed to prevent the progressive loss of RGC.

Beside RGC loss, microglial cell activation has been reported in the course of glaucoma in human patients (Neufeld, 1999; Wang et al., 2002) and animal models (Ebneter et al., 2010; Naskar et al., 2002; Wang et al., 2000). Upon activation, microglial cells express and secrete inflammatory proteins, which in turn lead to the production of nitric oxide and reactive oxygen species (Hanisch, 2002; Taylor et al., 2005), probably impairing RGCs function. Nevertheless, microglial cells have also beneficial function in neuropathological conditions, since they express neuroprotective molecules and are also necessary to remove debris caused by RGC death (reviewed in (Seitz et al., 2013)). Therefore, precise analysis and characterization of microglial cells in experimental glaucoma is of special interest with regard to new treatment approaches.

In addition to RGC degeneration and microglial activation, alterations of the retinal vasculature have been reported in the course of glaucoma in both patients (Findl et al., 2000; Gottanka et al., 2005; Michelson et al., 1996; Mitchell et al., 2005; Wang et al., 2010) and animal models (Almasieh et al., 2013). One important constituent of healthy microvessels, responsible for their proper function, are pericytes (PCs). These specialized pericapillary cells are located abluminal of endothelial cells on capillaries (Armulik et al., 2011) and in addition to their function in vessel stabilization and regulation, these cells are part of the blood–brain/retina-barrier (BBB/BRB) (Armulik et al., 2010). While single studies report increased leakage of the BRB in the optic nerve head (ONH) in glaucomatous patients (Arend et al., 2005; Grieshaber and Flammer, 2007), the tightness of the BRB in glaucoma is under debate. To unveil the role of retinal PC in glaucoma pathogenesis, we evaluated the PC coverage of microvessels and the tightness of retinal vessels in our model of OHT at different time points.

To get insight into the anatomical and functional changes of the hypertensive eye and to understand the pathology and etiology of RGC degeneration induced by glaucoma in humans, rodent glaucoma models are widely used (Vidal-Sanz et al., 2012). Elevated IOP in rodent models occurs spontaneously (John et al., 1998; Lindsey and Weinreb, 2005; Pang and Clark, 2007) or is created experimentally by surgical intervention. For the latter, several methods have been established to obstruct aqueous outflow: injections of hypertonic saline into the episcleral veins (Morrison et al., 1997), episcleral vein cauterization (Neufeld et al., 2002), laser coagulation of the anterior chamber angle (Ueda et al., 1998) or intracameral injections of foreign substances (i.e. latex microspheres) (Sappington et al., 2010).

The experiments of the present study were performed in an experimental glaucoma model (first described by Samsel et al. (Samsel et al., 2011)) using rats, injected with magnetic microbeads into the anterior chamber, leading to reliable and constant elevation of IOP.

To the best of the authors' knowledge, this is the first time the progression of RGC degeneration and microglial cell activation is evaluated over time in this acute model of OHT. Moreover, alterations in the retinal vasculature were investigated by analyzing the pericyte (PC) coverage of microvessels and the blood-retina-barrier tightness in ocular hypertension was tested by dextran injection.

2. Material and methods

2.1. Ocular hypertension model (OHT)

The injection of paramagnetic microbeads into the anterior chamber of the rat eye to obstruct aqueous outflow was first described by Samsel et al. (Samsel et al., 2011) and has been established and adapted in our laboratory. Male and female Brown Norway rats (5–15 month, n = 22) were anesthetized with a combination of ketamine/xylazine (100 mg/ml and 5 mg/ml i.p. Sigma–Aldrich, Vienna Austria). The treated eye received topical anesthesia with 4.0 mg oxybuprocain hydrochloride (0.4% Novain, Agepha), was disinfected with Povidon-Iod-complex solution (5% Betaisodona solution, Mundipharma), and rinsed with 0.9% NaCl. The 8 μm magnetic microbeads (COMPEL™ Magnetic, COOH modified, Bangs Laboratories Inc., USA) were washed multiple times in sterile 0.9% NaCl and sterilized by γ-irradiation (100 Gy, 30 min). The microbeads (MB) were injected into the anterior chamber of the left eye via a custom-built sharpened glass pipette (60–80 μm tip diameter, injection volume: 25 μl, concentration: 40.000 beads/μl, speed: 105 μl/min) and positioned into the iridocorneal angle with a handheld magnet. Application of a dexagenta-POS eye ointment (0.3 mg Dexamethason, 5.0 mg Gentamicin Sulfat, Chroma Pharma) prevented possible infections of the treated eye; to prevent desiccation of the contralateral untreated eye during the wakeup period, Vita-POS eye ointment (250 I.E./g Retinolpalmitat, Croma Pharma) was applied. All animal procedures were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the institutional animal care and use committee.

2.2. IOP measurements

Microbeads injection impedes aqueous drainage via occlusion of the trabecular meshwork and subsequently elevates the IOP. In order to minimize stress during IOP measurements in the awake