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Neuroprotective effect of minocycline in a rat model of branch retinal vein occlusion

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ABSTRACT

Branch retinal vein occlusion (BRVO) is the second most frequent retinal vascular disorder. Currently the first-line therapies for BRVO include anti-VEGF and dexamethasone implant treatment, however, with direct or indirect damage on retinal neurons, it has limited effect in improving patients visual acuity. Therefore, novel treatments with neuroprotective effect for BRVO retina were expected. Minocycline is a semisynthetic, broad spectrum tetracycline antibiotic with high penetration through the blood brain barrier. The neuroprotective effects of minocycline have been shown in various central nervous system (CNS) disease. Since both CNS and retina were composed of neurons and glials, it is reasonable to expect a neuroprotective effect by minocycline for BRVO retina. Therefore, the aim of the present study was to study whether minocycline has neuroprotective effect in branch retinal vein occlusion (BRVO) and the possible underlying molecular basis. We created BRVO in rats using laser photocoagulation. The animals were then randomly divided into 4 groups to evaluate the effect of minocycline: group A: minocycline 45 mg/kg intraperitoneal injection (i.p.), group B: minocycline 90 mg/kg i.p., group C: normal saline i.p., group D: sham injection. Fundus photography and fluorescein angiography (FA) were conducted. The changes in thickness of retinal layers were measured with optical coherence tomography (OCT) *in vivo*. We found that retinal edema occurred predominantly in the inner retinal layers. Intraperitoneal administration of minocycline significantly ameliorated retinal edema in the early stage of BRVO. We performed Full field Electroretinography (ffERG) to evaluate retinal function and found that the reduction of b wave amplitude decreased in the combined maximal response. The expressional levels of apoptosis related genes (*Bax*, *Bcl-2*) and inflammation related genes (*IL-1 β*, *TNF α*, *MCP-1* and *CCR2*) were measured by real-time PCR, the results showed that minocycline treatment upregulated *Bcl-2* expression and inhibits *TNF α* expression since early stage of BRVO. We also performed Hematoxylin–Eosin (HE) and immunostaining for Iba 1 (a microgilal marker), active caspase-3, Bax, Bcl-2, IL-1 β, TNF α and found that minocycline inhibits retinal microglial activation, prevents retinal ganglion cell loss, and inhibits retinal caspase-3 activation. Thus, our study indicates that systemic administration of minocycline ameliorates retinal edema and preserves retinal function in the early stage of BRVO possibly via inhibiting microglia activation and protecting RGC from apoptosis.

1. Introduction

Branch retinal vein occlusion (BRVO), with prevalence rates ranging from 0.3% to 1.1%, is the second most frequent retinal vascular disorder. The visual acuity of BRVO patients was usually poor at onset, and without intervention, clinically significant improvement beyond 20/40 was uncommon (Rogers et al., 2010).

Retinal edema and ischemia are the two major events in the pathogenesis of retinal vein occlusion. Thrombosis within a retinal vein obstructs the blood flow and increases the intraluminal pressure. The retinal perfusion pressure, which equals to the mean arterial blood pressure minus the venous pressure, is thus reduced,

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and the hypoxia–ischemia of retinal tissue occurs (Hayreh, 1994). A series of downstream cascades including inflammation and apoptosis process are then elicited and may further damage the retina (Kaur et al., 2008). Using electron microscope, major damages in each type of retinal neurons in a photothrombotic occlusion model of rat retinal vessels were observed Matini et al. (1997).

Currently the first-line therapies for BRVO include anti-VEGF and dexamethasone implant treatment (Badalà, 2008). However, VEGF is also a physiological factor (Ishida et al., 2003), indispensable for the maintenance of healthy neurons (Oosthuyse et al., 2001; Nishijima et al., 2007). Anti-VEGF therapy may threaten the neuroprotective effect by physical intraocular VEGF. The dexamethasone implant could cause elevation of intraocular pressure (Mayer et al., 2013), which may further damage the retinal neurons. Therefore, novel treatments with neuroprotective effect for BRVO retina were expected.

Minocycline is a semisynthetic, broad spectrum tetracycline antibiotic with high penetration through the blood brain barrier. Evidences from animal experiments and clinical data have shown the neuroprotective effects of minocycline in the treatment of central nervous system (CNS) disease beyond its antibiotic activity (Homsi et al., 2009; Pabreja et al., 2011; Plane et al., 2010; Zhao et al., 2011). Although the precise mechanisms regarding the neuroprotective effect of minocycline remain unclear, the proposed mechanisms include inhibition of inflammation and amelioration of apoptosis (Plane et al., 2010).

It has been reported that minocycline could inhibit the accumulation of microglia and the subsequent secretion of various inflammatory factors such as IL-1 β, IL-6 and TNF-α in CNS disease (Stirling et al., 2005; Kim and Suh, 2009). Minocycline can stabilize the mitochondrial membrane and prevents the release of cytochrome C into cytoplasm, thus inhibits the activation of caspase (Elewa et al., 2006; Teng et al., 2004). In addition, it is also reported that minocycline could inhibit apoptosis in caspase-independent way via upregulation of Bcl-2 (Wang et al., 2004).

Since both CNS and retina were composed of neurons and glials, and since evidence from animal experiments have shown that minocycline is effective in protecting the retina from light-induced photoreceptor damage (Zhang et al., 2004) and inhibiting apoptosis of photoreceptors in animal model of retinal detachment (Yang et al., 2009), it is reasonable to expect a neuroprotective effect by minocycline for BRVO retina. Therefore, the aim of the present study was to investigate whether minocycline had neuroprotective effect in a rat model of branch retinal vein occlusion and the possible underlying molecular basis of its action.

2. Materials and methods

2.1. Animals

All procedures were performed in accordance with the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research. Female adult Brown Norway rats (weight 200–250 g) were used. Prior to all procedures, the rats were anesthetized with an intraperitoneal injection of 1% sodium pentobarbital (45 mg/kg body weight). Eyes were topically anesthetized with Oxybuprocaine Hydrochloride solution (Santen Pharmaceutical Co Ltd), and pupils were dilated with Tropicamide Phenylephrine solution (Santen Pharmaceutical Co Ltd) prior to laser treatment and fundus examination.

2.2. Laser induced BRVO model

For each rat, one eye was randomly selected to produce photothrombotic BRVO with diode green laser, and the contralateral eye served as control. The rat model of retinal vein occlusion was established as previously described (Du et al., 2011; Hayreh et al., 2011). Retinal branch veins were irradiated 1.5–2.0 optic disk diameters (DD) away from the optic nerve with 532 nm diode laser (Novus spectra; Lumenis® Ltd, Santa Clara, CA). Laser parameters were as following: spot size 100 μm, power 180–240 mW, and exposure duration 0.4 s, 5–7 spots per vein. The rat fundus was viewed directly through contact lens (Ocular Instruments Inc., Bellevue, WA, USA). In a total, 3–4 veins were occluded in the BRVO procedure to generate an upper hemi-retinal occlusion. A total of 150 rats were studied.

2.3. Fundus photography and fluorescein angiography

To observe the evolution of BRVO and related retinal changes, fundus photography and fluorescein angiography (FA) were taken with spectralis HRA + OCT (Heidelberg Engineering, Inc.) at 1, 3, 5, 7 and 14 days after laser treatment. Sodium fluorescein (1%, 0.8 ml) was injected via the femoral vein and angiographs were recorded.

2.4. Experimental design and minocycline treatment

Animals after BRVO procedure were randomly divided into 4 groups: group A receiving daily intraperitoneal (i.p.) injection of minocycline hydrochloride at a dosage of 45 mg/kg (Sigma, St Louis, Missouri) beginning at 30 min after BRVO; group B receiving intraperitoneal injection of minocycline hydrochloride at a higher dosage (90 mg/kg); group C receiving injection of the same volume of normal saline; and group D receiving sham injection.

2.5. Optical coherence tomography

Optical coherence tomography (OCT) images were taken at 1, 3, 5, 7 and 14 days after laser treatment. Animals were placed on a platform and positioned below the OCT probe at a 30° angle to ensure that the scan beam was perpendicular toward the corneal surface. Artificial tears were applied every 2–3 min during alignment and imaging to prevent corneal dehydration and cataract formation. Simultaneous OCT and infrared reflectance (IR) imaging were taken with a spectralis HRA + OCT (Heidelberg Engineering, Inc.). During alignment and centering, the retina was directly visualized through the IR image. We used the optic nerve head as a landmark while taking OCT images. For each eye, OCT images were taken at 3 levels, that is 1, 2 and 3 optic disk diameters (DD) above the optic nerve head. At each level, 3 images were recorded: the image just above the optic disk was recorded as “middle”, and the images 1 field of view left and right to the “middle” one were recorded as “left” and “right”, respectively. Thus, images at 9 positions were recorded for each eye. All OCT measurements were performed by the same technician. Land marks such as distance from optic disk and morphology of the vasculature were used to ensure that OCT images were taken at the same position. All these procedures were performed in a masked fashion to avoid unintentional subjective bias.

Raw OCT images were quantitatively analyzed with Heidelberg Eye Explorer software. Retinal thickness (RT) measurements were performed manually, and for each OCT image, thickness of different retinal layers were measured: (1) retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL) (RNFL + GCL); (2) inner plexiform layer (IPL); (3) inner nuclear layer (INL); (4) outer nuclear layer (ONL); (5) retinal nerve fiber layer (RNFL) to inner nuclear layer (INL) (RNFL–INL); (6) retinal nerve fiber layer (RNFL) to photoreceptor layer (neurosensory retina).