Experimental Eye Research 143 (2016) 141–147

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Research article

The effect of intravitreal vascular endothelial growth factor on inner retinal oxygen delivery and metabolism in rats

Norman P. Blair [a, *], Justin Wanek [a], Pang-yu Teng [a, b], Mahnaz Shahidi [a]

[a] Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, 1855 West Taylor Street, Chicago, IL 60612, USA
[b] UCLA Radiological Sciences, Suite 650, 924 Westwood Boulevard, Los Angeles, CA 90024, USA

ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form
15 September 2015
Accepted in revised form 29 September 2015
Available online 28 October 2015

Keywords:
Vascular endothelial growth factor
Retina
Vasculature
Blood flow
Oxygen tension imaging
Oxygen delivery
Oxygen metabolism

ABSTRACT

Vascular endothelial growth factor (VEGF) is stimulated by hypoxia and plays an important role in pathologic vascular leakage and neovascularization. Increased VEGF may affect inner retinal oxygen delivery (DO_2) and oxygen metabolism (MO_2), however, quantitative information is lacking. We tested the hypotheses that VEGF increases DO_2, but does not alter MO_2. In 10 rats, VEGF was injected intravitreally into one eye, whereas balanced salt solution (BSS) was injected into the fellow eye, 24 h prior to imaging. Vessel diameters and blood velocities were determined by red-free and fluorescent microsphere imaging, respectively. Vascular PO_2 values were derived by phosphorescence lifetime imaging of an intravascular oxyphor. Retinal blood flow, vascular oxygen content, DO_2 and MO_2 were calculated. Retinal arterial and venous diameters were larger in VEGF-injected eyes compared to control eyes ($P < 0.03$), however no significant difference was observed in blood velocity ($P = 0.21$). Thus, retinal blood flow was greater in VEGF-injected eyes ($P = 0.007$). Retinal vascular PO_2 and oxygen content were similar between control and VEGF-injected eyes ($P > 0.11$), while the arteriovenous oxygen content difference was marginally lower in VEGF-injected eyes ($P = 0.05$). DO_2 was 950 ± 340 and 1380 ± 650 nL O_2/min in control and VEGF-injected eyes, respectively ($P = 0.005$). MO_2 was 440 ± 150 and 490 ± 190 nL O_2/min in control and VEGF-injected eyes, respectively ($P = 0.31$). Intravitreally administered VEGF did not alter MO_2 but increased DO_2, suggesting VEGF may play an offsetting role in conditions characterized by retinal hypoxia.

1. Introduction

Vascular endothelial growth factor (VEGF) is a signal protein that has potent mitogenic and survival activity for endothelial cells and powerful vascular permeability-enhancing properties. (Ferrara, 2004; Nishijima et al., 2007) Thus, it plays an important role in embryonic retinal vascular development, pathologic retinal vascular leakage, and neovascularization of the choroid, optic nerve, retina, and iris. (Ferrara, 2004) Accordingly, inhibitors of VEGF are now the treatments of choice for many cases of macular edema, (Brown et al., 2011; Campochiaro et al., 2011; Diabetic Retinopathy Clinical Research et al., 2010; Nguyen et al., 2010) choroidal neovascularization (Gragoudas et al., 2004; Rosenfeld et al., 2006) and neovascularization of the retina, optic nerve and iris. (Ernst et al., 2012; Grisanti et al., 2006; Martinez-Zapata et al., 2014).

Retinal hypoxia/ischemia, via hypoxia inducible factor (HIF), (Ozaki et al., 1999) is a major stimulator of VEGF. (Aiello et al., 1995; Miller et al., 1994; Pe'er et al., 1995) This suggests that VEGF may play a role in counteracting hypoxia and the injurious effects that supervene if the hypoxia is severe enough. There are several ways by which VEGF could mitigate hypoxic effects. First, by stimulating endothelial proliferation and vessel growth, VEGF could lead to better perfusion and alleviate retinal hypoxia. However, neovascularization often fails to vascularize ischemic tissue and instead can lead to deleterious effects such as hemorrhage and fibrosis. Furthermore, elevated VEGF over time can stimulate excessive endothelial proliferation that can occlude capillaries. (Hofman et al., 2001; Tolentino et al., 2002) Second, VEGF has been shown to have a survival role on retinal neural cells. (Saint-Geniez et al., 2008) Third, VEGF could increase retinal blood flow and oxygen delivery to the inner retina (DO_2). VEGF has been shown to cause retinal vasodilation, vascular tortuosity and increased blood flow in

* Corresponding author.
E-mail addresses: npblair@uic.edu (N.P. Blair), wanek_justin@yahoo.com (J. Wanek), pangyuteng@gmail.com (P.-y. Teng), mahnshah@uic.edu (M. Shahidi).

http://dx.doi.org/10.1016/j.exer.2015.09.018
0014-4835/

animals. (Ali Rahman et al., 2011; Ameri et al., 2007; Arana et al., 2012; Clermont et al., 1997; Ozaki et al., 1997) Several studies are available on retinal vascular changes after treatment of patients with VEGF inhibitors. A reduction in vessel diameters (Fontaine et al., 2011; Papadopoulou et al., 2009) and reductions in retrobulbar flow velocities have been observed. (Bonnin et al., 2010; Hosseini et al., 2012; Mete et al., 2010; Toklu et al., 2011) Nitta and coworkers found that the retinal blood flow measured by laser speckle flowgraphy decreased in patients treated for diabetic macular edema with an anti-VEGF agent. (Nitta et al., 2014) These observations imply that prior to treatment VEGF had increased retinal blood flow and DO_2. On the other hand, Fontaine and coworkers found no change in retinal blood flow by laser Doppler flowmetry after anti-VEGF therapy for age-related macular degeneration even though the retinal arterioles became narrower. (Fontaine et al., 2011) Barak and coworkers found increased blood velocity with the retinal function imager after anti-VEGF treatment, and they attributed this to suppression of vasodilation. (Barak et al., 2012) We have not found in the literature any measurements of DO_2 after the introduction of VEGF into the vitreous. Fourth, VEGF could down-regulate inner retinal oxygen metabolism (MO_2), as HIF does, (Kim et al., 2006) so that the tissue can adapt to mild reductions in oxygen availability. Reduced MO_2 might be expected to contribute to ischemic preconditioning and the suppression of cell death pathways, processes in which VEGF is thought to play a role. (Evans et al., 2008; Jin et al., 2001; Parcellier et al., 2003; Wick et al., 2002) However, we have not found any measurements in the literature on the effects of VEGF on inner retinal MO_2.

We hypothesized that VEGF increases DO_2, which could be advantageous in conditions characterized by hypoxia, but that it does not alter MO_2. We tested these hypotheses using the methods we have developed to measure DO_2 and MO_2 in rats. (Wanek et al., 2011, 2013, 2014).

2. Methods

2.1. Animals

Ten Long Evans pigmented rats (weight: 483 ± 127 g, mean ± SD) were used in the study. The rats were treated in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The rats were anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (5 mg/kg). Following the method of Miyamoto and co-workers, the right eye of each rat received an intravitreal injection of VEGF A (R & D Systems, Minneapolis, MN) while the fellow eye received vehicle (BSS) alone. (Miyamoto et al., 2000) The dose was increased from the concentration employed by Miyamoto et al. (50 ng in 5 μl) to 250 ng in 5 μl of BSS because of the extremely rapid clearance of VEGF from the vitreous as recently reported. (Lee et al., 2010) Data acquisition was performed 24 h after VEGF injection. The rats were reanesthetized with intraperitoneal injections of ketamine and xylazine. Additional injections of ketamine (20 mg/kg) and xylazine (1 mg/kg) were given to maintain anesthesia as required. Rats were ventilated mechanically with room air with the use of an endotracheal tube connected to a small animal ventilator (Harvard Apparatus, Inc., South Natick, MA). To monitor the animal's physiological condition, the femoral artery was cannulated and a catheter was attached to draw blood and connect a pressure transducer. Systemic arterial oxygen tension (P_aO_2), carbon dioxide tension (P_aCO_2), and pH were measured with a blood gas analyzer (Radiometer, Westlake, OH) 5–10 min after initiation of ventilation. Ventilation parameters, including the respiratory rate and minute volume, were adjusted until the P_aCO_2 was within the normocapnic range. (West, 2007) Hemoglobin concentration (HgB) was also measured with a hematology system (Siemens, Tarrytown, NY) from arterial blood. Blood pressure (BP) and heart rate (HR) were monitored continuously with a data acquisition system (Biopac Systems, Goleta, CA) linked to the pressure transducer.

Before imaging, the rat was placed in an animal holder with copper tubing from a water heater, which maintained the body temperature at 37 °C. The pupils were dilated with 2.5% phenylephrine and 1% tropicamide. A glass cover slip with 1% hydroxypropyl methylcellulose was applied to the cornea to eliminate its refractive power and prevent dehydration. For retinal vascular PO_2 imaging, an oxygen-sensitive molecular probe, Pd-porphine (Frontier Scientific, Logan, UT), was dissolved (12 mg/mL) in bovine serum albumin solution (60 mg/mL) and administered through the femoral arterial catheter (20 mg/kg). For retinal blood velocity imaging, 2-μm polystyrene fluorescent microspheres (Invitrogen, Grand Island, NY) were injected through the catheter. Typically, two to three injections of the microspheres were given, and each injection was approximately 0.4 mL (10^5 microspheres/mL).

2.2. Retinal hemodynamics imaging

Our previously described prototype blood flow imaging system (Wanek et al., 2011) was used for red-free and fluorescent microsphere imaging to assess venous blood vessel diameter and velocity, respectively. A slit lamp biomicroscope with standard light illumination (Carl Zeiss, Oberkochen, Germany) was equipped with a green filter (540 ± 5 nm; Edmund Optics, Barrington, NJ) for red-free retinal imaging, and a 488-nm diode laser (Melles Griot, Carlsbad, CA), coupled with an emission filter (560 ± 60 nm; Spectrotech, Inc., Saugus, MA) for fluorescent microsphere imaging. Images were captured with a high-speed electron multiplier charge coupled device camera (QImaging, Surrey, Canada). Red-free retinal images were obtained using the full resolution of the camera (1002 × 1004 pixels). For fluorescent images, the camera sensor was binned to maximize the frame rate to 108 Hz, allowing the motion of the microspheres to be resolved in time, but with lower spatial resolution (248 × 250 pixels). Multiple image sequences, each 5 s in duration, were recorded over several minutes.

Diameters of all individual retinal arteries (D_{Aind}) and veins (D_{Vind}) were measured from red-free images over a fixed vessel length (200 μm) that extended between approximately 300 and 500 μm from the center of the optic disk. D_{Aind} and D_{Vind} were obtained based on the average full width at half maximum of 12 intensity profiles perpendicular to the blood vessel axis. In control eyes 5.2 ± 1.0 arteries and 5.2 ± 1.1 veins were measured, whereas 5.5 ± 1.1 arteries and 5.4 ± 1.0 veins were included from VEGF-injected eyes. For each rat, a mean arterial (D_A) and venous diameter (D_V) was calculated from all D_{Aind} and all D_{Vind} values, respectively. Blood velocity in all individual veins (V_{ind}) was measured by manually tracking displacements of the microspheres over time, following our previously reported methodology. (Wanek et al., 2011) Typically, three to five image sequences were analyzed to derive V_{ind}, which was determined by averaging 20–30 microsphere velocity measurements in each vein. The number of measurements used to obtain each V_{ind} was contingent on the number of microspheres that could be visualized in the image sequences. In each rat, a mean velocity (V) was derived from all V_{ind} measurements. V was measured in veins because they are less affected by pulsation and have larger diameters as compared with arteries. Blood flow in each major vein was calculated from D_{Vind} and V_{ind} measurements ($V_{ind}{}^{*}\pi^{*}D^2_{Vind}/4$) and summed over all veins to provide a measure of the total venous blood flow in the retinal circulation (F) in that animal. Since the retinal circulation is considered to be an end-artery system, (Levin and Adler, 2011) the venous blood flow was taken to be equal to the total arterial or total