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Correspondence of retinal thinning and vasculopathy in mice with oxygen-induced retinopathy

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ABSTRACT

The aberrantly vascularized peripheral retina in retinopathy of prematurity (ROP) may be associated with visual field constriction, retinal dysfunction, and abnormalities in retinal thickness which is commonly assessed by spectral domain optical coherence tomography (SDOCT). However, due to the limitation of SDOCT for peripheral retinal imaging, retinal thickness in avascular peripheral retina in ROP has not been evaluated. Oxygen induced retinopathy (OIR) in mice has features of vasculopathy similar to those in human ROP. These features occur in the posterior retina and thereby are accessible by standard imaging methods. The purpose of the current study was to determine the correspondence between abnormalities in retinal thickness and vasculopathy in neonatal OIR mice by simultaneous SDOCT imaging and fluorescein angiography (FA). Newborn mice ($N = 19$; C57BL/6J strain) were exposed to 77% oxygen from postnatal day 7 (P7) to P12. Age-matched control mice ($N = 12$) were raised in room air. FA and SDOCT were performed in mice between P17 and P19 to visualize retinal vasculature and measure retinal thickness, respectively. Retinal thickness measurements in vascular regions of interest (ROIs) of control mice, and in hypovascular and avascular ROIs of OIR mice were compared. In control mice, FA showed uniformly dense retinal capillary networks between major retinal vessels and retinal thickness of vascular ROIs was 260 ± 7 μm ($N = 12$). In OIR mice, FA displayed hypovascular regions with less dense and fewer capillaries and avascular regions devoid of visible capillaries. Retinal thickness measurements of hypovascular and avascular ROIs were 243 ± 21 μm and 209 ± 11 μm ($N = 19$), respectively. Retinal thickness in hypovascular and avascular ROIs of OIR mice was significantly lower than in vascular ROIs of control mice ($p \leq 0.01$). Likewise, retinal thickness in avascular ROIs was significantly lower than in hypovascular ROIs ($p < 0.001$). Retinal thinning in hypovascular and avascular regions may be due to arrested retinal development and/or ischemia induced apoptosis.

1. Introduction

Retinopathy of prematurity (ROP) can be associated with visual deficits related to abnormal vascular development. Compared to normal preterm eyes, eyes with ROP have visual field constriction with or without treatment of the peripheral, aberrantly vascularized retina (Quinn et al., 1996). Reduction in visual field extent may be caused by ROP or cryotherapy and laser treatments of avascular peripheral retina (O'Connor et al., 2007; Quinn et al., 2011), but it is not well understood how visual field constrictions may decrease patient functional ability. Patients with mild and severe ROP have deficits in rod photoreceptor and post-receptor sensitivity as demonstrated by electroretinogram studies (Harris et al., 2011). While post-receptor rod retinal sensitivity improves in mild ROP, post-receptor recovery is reduced in retinas with severe ROP, likely due to abnormalities in the post-receptor neural retina and its vascular supply (Harris et al., 2011). Related to ROP severity, the peripheral avascular retina in the acute phase of ROP may develop abnormal visual function even if later vascularized during ROP regression. This vulnerable, aberrant vascularized peripheral retina has been well studied with fluorescein angiography (FA) but has not been adequately imaged with spectral domain optical coherence

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tomography (SDOCT) due to limitations in imaging the peripheral retina beyond Zone 1 (Maldonado et al., 2012). Improved understanding of retinal vasculature and layer morphology in ROP is critical for determining the effects of ROP and possible treatments on retinal structure, function, and visual prognosis (Fulton et al., 2009; Harris et al., 2011).

Oxygen induced retinopathy (OIR) in the mouse has been used to model human ROP and has demonstrated ROP-related features including avascularity, vessel dilation and tortuosity, and neovascularization more centrally compared to the same features in the peripheral retina of human ROP (Smith et al., 1994). OIR studies have traditionally used retinal flat mounts to determine vascular patterns and histologic sections for evaluation of retinal morphology in different enucleated eyes (Smith et al., 1994). This separate assessment of retinal vasculature and morphology has not hindered research progress, but it has made it impossible to directly relate spatial variations of retinal layer architecture to en face vascular abnormalities. Accordingly, in prior OIR studies, any change in histology related to abnormal vasculature has often only been assumed by inference among different eyes rather than direct evidence from the same eyes.

More recently, FA in living OIR mice has confirmed prior retinal flat mount findings of vascular changes (Mezu-Ndubuisi et al., 2013; Nakao et al., 2013). Furthermore, the posterior location of retinal pathology in OIR, unlike human ROP, may be imaged simultaneously with FA and SDOCT. In the present study, FA and SDOCT imaging techniques were utilized to provide correspondence of retinal vascular and thickness abnormalities in the same locations of the same eyes of living OIR mice.

2. Experimental procedures

2.1. Animals

All experimental procedures were approved by the Animal Care Committee of the University of Illinois at Chicago and were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. The procedures have been previously reported (Mezu-Ndubuisi et al., 2013). Neonatal wild type mice (C57BL/6J) were separated into two experimental groups (OIR and control). Control mice were raised in room air throughout the experiment. Using an established model (Smith et al., 1994), the OIR mice were exposed to 77% oxygen in a hyperoxia chamber (Biospherix Ltd., Redfield, NY) for 5 days starting from postnatal day 7 (P7) which induced vaso-obliteration. At P12, the mice were returned to room air which triggered vascular regrowth due to the relative hypoxia. Imaging was performed in control and OIR mice at P17, P18, or P19, when pronounced vasculopathy features were present. The body weights of the control and OIR mice were 8.2 ± 2.6 g (mean $\pm$ SD; $N = 12$) and 6.1 ± 1.3 g ($N = 19$), respectively. Prior to imaging, mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (5 mg/kg) and pupils were dilated. For FA, an intraperitoneal injection of 10% fluorescein sodium (AK-FLUOR®, Akorn, Decatur, IL) was administered at a dose of 100 mg/kg.

2.2. Imaging and image analysis

FA and SD-OCT imaging were performed simultaneously with a commercially available instrument (Spectralis, Heidelberg Engineering, Heidelberg, Germany) in one eye of each mouse. FA images were acquired with a field of view of approximately 5 optic disk (OD) diameters. Images were obtained nasal or temporal to the OD because the instrument's range of motion was greater horizontally (nasal and temporal) than vertically (superior and inferior). In the same imaged area, SD-OCT volume data were acquired consisting of 31 raster B-scans (1536×496 pixels).

FA images in control mice showed a uniformly dense capillary network (vascular), while in OIR mice, the capillary network was less dense (hypovascular), and in some regions, no capillary network was visible (avascular). Based on visualization of the vasculature in FA images, in each control mouse, one region of interest (ROI) was selected between major retinal blood vessels that displayed a uniformly dense capillary network (vascular). In each OIR mouse, two ROIs (hypovascular and avascular) were selected. Hypovascular ROIs had fewer visible and often enlarged capillaries compared to the capillary network of control mice, and avascular ROIs were devoid of visible capillaries. In 19 of 25 OIR mice that were imaged, both avascular and hypovascular ROIs were clearly discerned and therefore data from these mice were selected to permit paired comparisons. In each of 12 control mice, one vascular ROI was selected. The number of control mice imaged at P17, P18, and P19 was 6, 3, and 3, respectively, while the number of OIR mice imaged at P17, P18, and P19 was 7, 5, and 7, respectively.

Retinal thickness maps were generated automatically using the instrument's software by measuring the distance between the internal limiting membrane (ILM) and retinal pigment epithelium (RPE) in each SD-OCT B-Scan. Retinal thickness maps were visually inspected for regions of abrupt change in thickness due to segmentation errors by the software. B-scans that traversed these regions were examined for ILM or RPE segmentation errors, which were corrected manually. An average retinal thickness was obtained in the central subfield (approximately one disk area in mice) of the Early Treatment Diabetic Retinopathy Study target, which was manually placed on each ROI.

Twelve vascular ROIs (6 nasal and 6 temporal) were identified in 12 control mice and 19 hypovascular and 19 avascular ROIs (9 nasal and 10 temporal) were identified in 19 OIR mice. In OIR mice, hypovascular and avascular ROIs were on the same side of the OD. Since normal spatial variations in retinal thickness may be present, to ensure thickness measurements were obtained from similar retinal regions of control and OIR mice, distances of vascular, hypovascular, and avascular ROIs from the OD center were calculated and statistically compared. Furthermore, similarity of normal retinal thickness between nasal and temporal vascular ROIs at equivalent distances from the OD was statistically verified.

2.3. Data analysis

Mean retinal thickness measurements obtained at different postnatal ages (P17, P18, and P19) were compared using one-way analysis of variance. Mean distances from the OD and retinal thickness between hypovascular (or avascular) and vascular ROIs were compared using unpaired Students *t*-test. Mean distances from the OD and retinal thickness between hypovascular and avascular ROIs were compared using paired Students *t*-test. Mean distances from OD and retinal thickness between nasal and temporal vascular ROIs were compared using unpaired Students *t*-test. Statistical significance was accepted at $p < 0.05$.

3. Results

An example of an FA image acquired in a control mouse in a region nasal to the OD is shown in Fig 1A. The FA image revealed a uniformly dense capillary network and normal major retinal blood vessels. The retinal thickness map generated in the same control mouse and overlaid on the FA image is displayed in Fig 1B. The thickness map was relatively uniform with minor variations near the OD. Retinal thickness in one selected vascular ROI in this control mouse was 266 μm.