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Regional heterogeneity of endothelial cells in the porcine vortex vein system

Priscilla Ern Zhi Tan [a,b], Paula K. Yu [a,b], Stephen J. Cringle [a,b], William H. Morgan [a], Dao-Yi Yu [a,b,*]

[a] Centre for Ophthalmology and Visual Science, The University of Western Australia, Lions Eye Institute, 2 Verdun Street, Nedlands, Perth, Western Australia 6009, Australia
[b] The ARC Centre of Excellence in Vision Science, The University of Western Australia, Lions Eye Institute, 2 Verdun Street, Nedlands, Perth, Western Australia 6009, Australia

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ABSTRACT

Purpose: The aim of this study was to investigate whether region-dependent endothelial heterogeneity is present within the porcine vortex vein system.
Methods: The superior temporal vortex vein in young adult pig eyes were dissected out and cannulated. The intact vortex vein system down to the choroidal veins was then perfused with labels for f-actin and nucleic acid. The endothelial cells within the choroidal veins, pre-ampulla, anterior portion of the ampulla, mid-ampulla, posterior portion of the ampulla, post-ampulla, intra-scleral canal and the extra-ocular vortex vein regions were studied in detail using a confocal microscopy technique. The endothelial cell and nuclei length, width, area and perimeter were measured and compared between the different regions.
Results: Significant regional differences in the endothelial cell and nuclei length, width, area and perimeter were observed throughout the porcine vortex vein system. Most notably, very narrow and elongated endothelia were found in the post-ampulla region. A lack of smooth muscle cells was noted in the ampulla region compared to other regions.
Conclusions: Heterogeneity in endothelial cell morphology is present throughout the porcine vortex vein system and there is a lack of smooth muscle cells in the ampulla region. This likely reflects the highly varied haemodynamic conditions and potential blood flow control mechanisms in different regions of the vortex vein system.

Introduction

Endothelial phenotypic heterogeneity is a core property of the endothelium and has been described at the level of cell morphology, function, gene expression, and antigen composition (Aird, 2003, 2005, 2007a, 2007b, 2012; Aird and Rosenberg, 1997; Regan and Aird, 2012). Endothelial phenotypes are organ-specific and site-specific within the same organ, and can be indicative of their normal physiological or pathophysiological states (Chiu and Chien, 2011).

Vascular endothelial cells line the inner wall of all vasculature and are continually exposed to haemodynamic forces. They receive information through active transport, direct permeation, or indirect changes in smooth muscle and other vessel wall components (Cines et al., 1998). An immediate response to protection against haemodynamic disruptions is achieved by a sudden transformation of the endothelium to a vasoconstrictive, pro-coagulant, and pro-inflammatory state, resulting in various changes in its structure and behaviour (Cines et al. 1998). Research has shown that numerous vascular diseases are due to these changes in the endothelial intracellular cytoskeleton under stress, with resultant excessive and prolonged endothelial activation leading to dysfunction and pathogenesis (Fryer et al., 1993; Henry, 1994; McGorisk and Treasure, 1996; Ross, 1993; Schmidt et al., 1994; Steinberg et al., 1996). As abnormal changes in haemodynamics affect blood flow pattern (Grabowski and Lam, 1995; Kumar et al. 1998; Yu et al., 2012) and endothelial cells have a critical role in vascular biology and pathophysiology of disease processes (Chiu and Chien, 2011; Chiu et al., 1998, 2009), it is important to identify any patterns which may point to preclinical vascular disease.

The venous system is more complex than the arterial system in many respects (Meissner et al., 2007) and venous diseases are ten times more frequent than arterial diseases (Monos et al., 1995). Although previous studies have focused mostly on large vessels and cultured cells (Bhuyan et al., 1991; Chiu and Chien, 2011; Chiu et al., 2009), and demonstrated that endothelial structural remodelling occurs in response to haemodynamic shear stress (Ando and Yamamoto, 2009, 2011; Reinhart-King et al., 2008; Simmons et al., 2012), recent studies have identified location specific phenotypic differences of endothelium at the level of venous microvasculature. Studies of endothelial cell morphology in the central and branch retinal veins in human donor eyes (Kang et al., 2011; Yu et al., 2012) have found site-specific changes at

Abbreviations: STVV, superior temporal vortex vein; ITVV, inferior temporal vortex vein.

* Corresponding author at: Centre for Ophthalmology and Visual Science and the ARC Centre of Excellence in Vision Science, The University of Western Australia, 2 Verdun Street, Nedlands, Western Australia 6009, Australia. Fax: +61 8 9381 0700.
E-mail addresses: priscilla.ez.tan@gmail.com (P.E.Z. Tan), paula.yu@uwa.edu.au (P.K. Yu), cringle@cyllene.uwa.edu.au (S.J. Cringle), BillMorgan@lei.org.au (W.H. Morgan), dyyu@lei.org.au (D.-Y. Yu).

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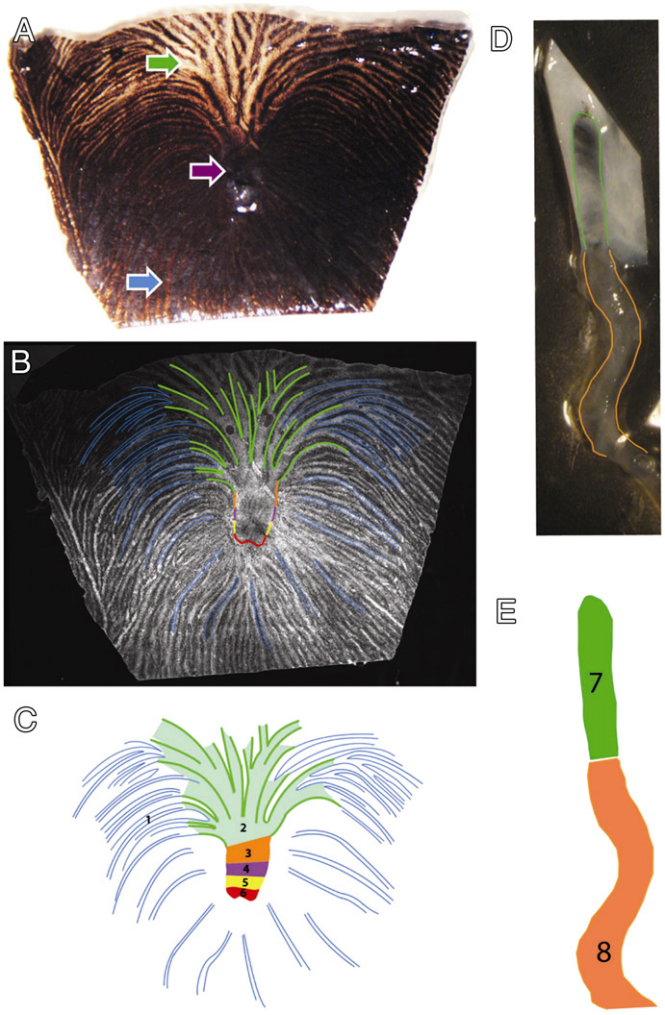

Fig. 1. Regions of study. (A) and (D) are photographic images of the dissected choroidal and scleral portions of a porcine superior temporal vortex vein system. The images have been oriented with the top of the images pointing anteriorly. (A) is a photographic image of the intra-ocular choroidal regions taken from the scleral aspect with the ampulla facing up. The green arrow points to the large, anteriorly draining choroidal vessels and pre-ampulla which appear as clear tubular spaces in amongst the heavily pigmented extravascular choroidal tissue. The blue arrow points to the smaller but more numerous choroidal vessels that drain the posterior globe. The purple arrow corresponds to the mid-ampulla and the cut edge where the vortex vein meets the sclera can be seen in the middle of this image as a reflective bulb. (B) is the fluorescence image of (A) labelled for f-actin. Choroidal vessels are clearly visible as fluorescent, white curvy lines running towards the ampulla. The regions studied were outlined using different colours. (C) is a schematic drawing of the corresponding regions studied with a solid colour fill. Note that not all the choroidal venules have been outlined in B or C as they were too numerous. The numbering for the regions corresponds as follows: 1 = Choroidal vein (blue); 2 = Pre-ampulla (pale green); 3 = Anterior portion of the ampulla (orange); 4 = Mid-ampulla (purple); 5 = Posterior portion of the ampulla (yellow); 6 = Post-ampulla (red). (D) is a photographic image of a bisected superior temporal vortex vein inside the sclera and as it exits the sclera. The upper half of the image shows the intra-scleral portion of the single exiting vessel within white scleral tissue outlined in green. The curvy, opaque lower half of the image contains the extra-ocular vortex vein outlined in orange. (E) is the schematic drawing of these two portions with a solid colour fill. 7 = Intra-scleral canal (dark green); 8 = Extra-ocular vortex vein (orange).

these locations known to be vulnerable to venous occlusive diseases. It is therefore of interest to study other site-specific venous regions within the eye for other possible locations of endothelium vulnerability.

The choroidal circulation has a high blood flow rate (Alm, 1992) and plays a vital role in the maintenance of the outer retina. The vortex veins are a major drainage pathway for the choroidal circulation and are located in each quadrant of the eyeball near the equator (Kutoglu et al., 2005). In addition to nearly all the venous drainage of the choroid, the vortex veins also drain the venous blood from the anterior portion of the eye. Numerous choroidal veins converge into a sacculated dilatation called the ampulla before entering the sclera, forming a unique anatomical structure known as the vortex vein system (Hogan et al., 1971; Kaufman and Alm, 2003). A culmination of high flow rates and large volumes of blood through a geometrically complicated vortex vein system has the potential to induce unusual flow patterns and haemodynamic forces (Bill, 1962), exposing vascular endothelial cells to various haemodynamic forces in different regions of the vortex vein system. It is possible that different haemodynamic pressure gradients and stressors such as shear and turbulent forces within the vortex vein system may affect the endothelium and present with special geographic patterns for specific regions. Exposure to such complicated haemodynamics may predispose the vortex vein endothelium to pathological phenotype, followed by vascular disease. It is therefore important that a detailed investigation of the endothelial cell intracellular structures within the vortex vein system of the eye be conducted in order to understand its functional role and determine whether there could be pathological changes with age or disease.

The purpose of this study is to determine if there are region-dependent differences in the endothelial cell morphologies within the vortex vein system. We propose that there would be haemodynamic changes due to special vascular distribution patterns, which would thus lead to a regional heterogeneity of endothelial cells. We have chosen the porcine eye as our baseline animal model due to the similarity of the porcine eye in anatomy and physiology to the young normal human eye, and will thus enable good comparisons to future studies of human