Experimental Eye Research 132 (2015) 249–258

Contents lists available at ScienceDirect

Experimental Eye Research

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

Quantitative study of the microvasculature and its endothelial cells in the porcine iris

Hongfang Yang [a, b, c], Paula K. Yu [a, b], Stephen J. Cringle [a, b], Xinghuai Sun [c, **], Dao-Yi Yu [a, b, *]

[a] Centre for Ophthalmology and Visual Science, The University of Western Australia, Perth, Australia
[b] Lions Eye Institute, The University of Western Australia, Perth, Australia
[c] Department of Ophthalmology, EENT Hospital, Fudan University, Shanghai, China

ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form
6 February 2015
Accepted in revised form 9 February 2015
Available online 11 February 2015

Keywords:
Iris microvasculature
Endothelium
Micro-perfusion
Vascular biology
Microvascular networks

ABSTRACT

The roles of the iris microvasculature have been increasingly recognised in the pathogenesis of glaucoma and cataract; however limited information exists regarding the iris microvasculature and its endothelium. This study quantitatively assessed the iris microvascular network and its endothelium using intraluminal micro-perfusion, fixation, and staining of the porcine iris. The temporal long posterior ciliary artery of 11 isolated porcine eyes was cannulated, perfusion-fixed and labelled using silver nitrate. The iris microvasculature was studied for its distribution, orders and endothelial morphometrics. The density of three layers of microvasculature was measured. Endothelial cell length and width were measured for each vessel order. The iris has an unusual vascular distribution which consisted of abundant large vessels in the middle of the iris stroma, branching over a relatively short distance to the microvasculature located in the superficial and deep stroma as well as the pupil edge. The average vascular density of the middle, superficial, and deep layers were 38.9 ± 1.93%, 10.9 ± 1.61% and 8.0 ± 0.79% respectively. Multiple orders of iris vessels (capillary, 6 orders of arteries, and 4 orders of veins) with relatively large capillary and input arteries (319.5 ± 25.6 μm) were found. Significant heterogeneity of vascular diameter and shape of the endothelia was revealed in different orders of the iris vasculature. Detailed information of topography and endothelium of the iris microvasculature combined with unique structural features of the iris may help us to further understand the physiological and pathogenic roles of the iris in relevant ocular diseases.

1. Introduction

The iris vasculature is the major source of oxygen and nutrients supplying the anterior segment. The anterior segment is a highly specialized structure consisting of avascular tissues such as the cornea, lens, and aqueous humour (Tasman and Jaeger, 2005). It is thought that the iris vasculature not only has a role in maintaining intraocular homeostasis, it is also likely to have a role in the sequential survival as well as function of the cells in the anterior segment when the iris vasculature undergoes pathological changes.

The impact of the iris vasculature on the anterior segment is not well defined. Recent non-invasive imaging techniques such as ultrasound biomicroscopy and optical coherence tomography have provided some insights suggesting a possible role in the pathogenesis of angle closure glaucoma (Wang et al., 2010). Significant changes in iris volume have been observed using these imaging devices and postulated to be a major pathogenic factor for primary angle closure glaucoma (Aptel et al., 2012; Baskaran et al., 2013; Mak et al., 2013; Oyster, 2000; Quigley, 2009; Seager et al., 2014). The dynamic processes leading to changes in iris and choroid volume could have a more significant and mechanistic effect to its surrounding structures than a mere anatomical observation (Quigley, 2009). Hence the mechanisms involved in iris volume change as well as the consequence from the iridal volume change could well be an important pathogenic factor in glaucoma.

Secondly, both clinical and experimental studies demonstrated significant oxygen gradients are present normally in the anterior

* Corresponding author. Centre for Ophthalmology and Visual Science, The University of Western Australia, Nedlands, WA 6009, Australia.
** Corresponding author.
E-mail addresses: xhsun@smhu.edu.cn (X. Sun), dyyu@cyllene.uwa.edu.au (D.-Y. Yu).

http://dx.doi.org/10.1016/j.exer.2015.02.006
0014-4835/

chamber. We now know that changes in oxygen gradients and oxidative stress are major pathogenic factors for cataract and glaucoma (Barbazetto et al., 2004; Holekamp et al., 2005; Shui et al., 2006). The lens is a structure which normally has a relatively low oxygen level (Barbazetto et al., 2004; Holekamp et al., 2005; Shui et al., 2006). Exposure of the lens to elevated intraocular oxygen and the subsequent increase in reactive oxygen species (ROS) (Barbazetto et al., 2004; Truscott, 2005) is a risk factor for nuclear cataracts (Barbazetto et al., 2004; Holekamp et al., 2005; Siegfried et al., 2010). It has been said that increased oxidation may be the hallmark of age-related nuclear cataract (Truscott, 2005). Recent reports that vitrectomy and cataract surgery can increase oxygen delivery to the outflow pathway and directly increases oxidative stress (Chang, 2006; Luk et al., 2009; Siegfried et al., 2010). Oxidative stress may also present an important pathogenic step in primary open-angle glaucoma by inducing human trabecular meshwork degeneration, favouring an intraocular pressure (IOP) increase, thus priming the glaucoma pathogenic cascade (Izzotti et al., 2009; Sacca et al., 2005). The iris microvasculature being the predominant source of oxygen in the region most likely plays a critical role in maintaining oxygen homeostasis.

Thirdly, differential protein concentration is known to exist in the aqueous humour and ciliary process stroma. The protein concentration in the aqueous humour (50 mg/dl) is less than 1% of that in plasma (8 g/dl) (Smith et al., 1985) whereas that in ciliary process stroma is 74% of that in plasma (Bill, 1968). This is likely a consequence of differential permeability to protein in the iridal and ciliary vasculature. Evidence has been found where intravascular horse radish peroxidase appeared in the iridal stroma having leaked from ciliary body but not from the iridal vasculature (Freddo et al., 1990). Similarly, transport in the reverse direction back into the blood stream found only anionic molecules are transported back via plasmalemmal vesicles of endothelial cells in both the retinal and iridal vasculature with cationic molecules not returned by this pathway (Raviola and Butler, 1985). This further suggests heterogenic property of the endothelium in the iridal and ciliary vasculatures. If we are to assume that open communications exist freely between the interstitial spaces of the iris and the anterior chamber, then it may be further speculated that the iris microvasculature and its endothelium holds the control mechanism to maintain homeostasis in the anterior segments. In recent decades, the vascular endothelium has emerged not merely as an inert barrier but also an active signal transducer for circulating influences as well as the key regulator of material exchange (Deanfield et al., 2007). Previous studies using corrosion casts had provided some qualitative information on the iris vasculature (Funk and Rohen, 1987, 1990; May, 2011; Ninomiya et al., 2008; Olver and McCartney, 1989a, 1989b; Risco and Nopanitaya, 1980; Rohen and Funk, 1994). Whilst limited information is available on the iridal endothelium both in the structural and functional aspects, this study provided some detail information on iridal endothelium morphometrics and vessel density. This information could help us to further understand and investigate the structural difference in iridal endothelium as well as in considering the role of the iris microvasculature in normal and disease conditions.

2. Materials and methods

Pig eyes were obtained from a local abattoir. Following enucleation, the eyes were placed in a sealed bottle of oxygenated Krebs solution and kept on ice during transfer to the laboratory (~60 min). Eleven eyes with sufficiently long temporal long posterior ciliary arteries (LPCA) and lightly pigmented irises were selected for this study. All procedures conformed to the EU Directive 2010/63/EU for animal experiments.

The dissection, cannulation and arterial perfusion in isolated eyes are fully described in our previous publications (Townsend et al., 2006; Yu et al., 2003). Similar techniques were used in the present study and will be briefly described. Before perfusion, the nasal LPCA and all other temporal small arteries were tied off with 9-0 nylon sutures. Details of method of perfusion staining of ocular microvasculature in our lab have been published previously (Yu et al., 2010b) and were modified for silver nitrate labelling of the iris microvasculature. Briefly, the pig eye was placed temporal side facing up in an eye holder, the temporal LPCA was cannulated using glass micropipettes with tip sizes of 290 to 320 μm, and secured in place by a 9-0 nylon suture. A flow rate of 200 μl/min was delivered using a syringe pump (model 22; Harvard Apparatus, South Natick, MA) throughout the whole perfusion sequence except for the silver staining part. Firstly, residual blood was washed out with filtered oxygenated Ringer's solution containing 1% bovine serum albumin for 30 min. This was followed by filtered 4% paraformaldehyde in 0.1 M phosphate buffer perfused for 5 min. Silver nitrate labelling of cell border was based on a modified protocol of P Baluk's method (Baluk et al., 1997). Five solutions were perfused in rapid succession: 0.9% NaCl 2 min; 5% glucose (2 ml hand pushed in 2 min); 1% $AgN0_3$ (1 ml hand pushed over 1 min); 5% glucose (1–1.5 ml hand pushed for 1 min); and 4% paraformaldehyde pump perfused for 3 min. The anterior segments were subsequently immersion fixed in 4% paraformaldehyde overnight. The rationale of using silver nitrate staining in this study was to obtain clear morphology of endothelial cells from the iris stroma (Baluk et al., 1997).

The iris was carefully dissected out from the root connected to the ciliary body. To bleach the pigments covering the surfaces of the iris, irises were kept in 10% H_2O_2 in 0.05 M Tris HCl buffer, pH 7.6, for up to 48 h at room temperature. After bleaching, the iris was exposed to bright light to visualise the silver deposit at cell borders. The iris was then immersed in RapiClear® 1.47 (Sunjin Lab, Taiwan) for 2 h for clearing.

The intact cleared iris was flat mounted for light microscopy imaging using the Nikon i90 and NIS basic software (v.3.8, Nikon). Low magnification Z-stack images were taken to a depth of 210 μm using a ×4 objective lens and up to 250 μm using a ×10 objective lens. This enabled three-dimensional data collection on the distribution of iris microvasculature. Specific vascular segments were examined using higher power objective lenses (×40 plan apochromatic oil lenses) with each z-stack consisting of a depth of optical sections collected at 1 μm increments along the z-plane, for detailed structures in the vascular endothelium and smooth muscle cells. Z-projection images were made using Image Pro Plus (v.7.0, Media Cybernetics, Inc.) and sketches of endothelium were made to outline the cell shape using Adobe Illustrator CS4.

Topologic description of vessel trees by the Horton–Strahler nomenclature was performed in this study. The Horton–Strahler scheme starts at the capillary level and proceeds centripetally, and the convergence of two equal order branches gives one increase in the order of vessels (i.e. where two capillaries joined together to form a first-order arteriole (A-1) and two A-1 arterioles joined together to form a second-order arteriole (A-2)).

Morphometric measurements of vessel diameter, endothelial cell length and width were made NIS basic software (v.3.8, Nikon). Arteries are recognized as vessels with spindle endothelial cells and thick smooth muscle cells wrapping around; whereas veins have polygonal shaped endothelial cells and connected to arteries by capillaries. As iris microvessels are not uniform in their diameters along their lengths, every vessel of each order was measured at five positions and averaged for diameter measurement. Endothelial cell length measurements were taken as the longitudinal distance from the upstream pole to the downstream pole. Endothelial cell widths measurements were taken as the greatest perpendicular width to