Experimental Eye Research 140 (2015) 106–116

Contents lists available at ScienceDirect

Experimental Eye Research

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

Intracellular cytoskeleton and junction proteins of endothelial cells in the porcine iris microvasculature

Hongfang Yang [a, b, c], Paula K. Yu [a, b], Stephen J. Cringle [a, b], Xinghuai Sun [c, d, e, **], 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 & Visual Science, Eye & ENT Hospital, State Key Laboratory of Medical Neurobiology, Institutes of Brain Science, Shanghai Medical College, Fudan University, Shanghai, 200032, China
[d] Key Laboratory of Myopia, Ministry of Health, Fudan University, China
[e] Shanghai Key Laboratory of Visual Impairment and Restoration, Fudan University, China

ARTICLE INFO

Article history:
Received 27 May 2015
Received in revised form
21 August 2015
Accepted in revised form 27 August 2015
Available online 31 August 2015

Keywords:
Iris microvasculature
Endothelium
Micro-perfusion
Cytoskeleton
Junction proteins
Claudin-5
VE-cadherin

ABSTRACT

Recently we reported studies of the iris microvasculature and its endothelial cells using intra-luminal micro-perfusion, fixation, and silver staining, suggesting that the iris vascular endothelium may be crucial for maintaining homeostasis in the ocular anterior segment. Here we present information regarding the intracellular structure and cell junctions of the iris endothelium.

Thirty-seven porcine eyes were used for this study. The temporal long posterior ciliary artery was cannulated to assess the iris microvascular network and its endothelium using intra-luminal micro-perfusion, fixation, and staining with phalloidin for intracellular cytoskeleton f-actin, and with antibodies against claudin-5 and VE-cadherin for junction proteins. Nuclei were counterstained with Hoechst.

The iris was flat-mounted for confocal imaging. The iris microvasculature was studied for its distribution, branch orders and endothelial morphometrics with endothelial cell length measured for each vessel order. Our results showed that morphometrics of the iris microvasculature was comparable with our previous silver staining. Abundant stress fibres and peripheral border staining were seen within the endothelial cells in larger arteries. An obvious decrease in cytoplasmic stress fibres was evident further downstream in the smaller arterioles, and they tended to be absent from capillaries and veins. Endothelial intercellular junctions throughout the iris vasculature were VE-cadherin and claudin-5 immunopositive, indicating the presence of both adherent junctions and tight junctions between vascular endothelial cells throughout the iris microvasculature. Unevenness of claudin-5 staining was noted along the endothelial cell borders in almost every order of vessels, especially in veins and small arterioles.

Our results suggest that significant heterogeneity of intracellular structure and junction proteins is present in different orders of the iris vasculature in addition to vascular diameter and shape of the endothelia. Detailed information of the topography and intracellular structure and junction proteins of the 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 vasculature in relevant ocular diseases.

1. Introduction

The iris is the most anterior portion of the uvea, lying between the anterior and posterior chambers (Hogan et al., 1971). The anterior segment consists of avascular tissues such as the cornea, lens, and aqueous humour (Tasman and Jaeger, 2005). The role of the iris vasculature in maintaining intraocular homeostasis is an

* Corresponding author. Centre for Ophthalmology and Visual Science, The University of Western Australia, Nedlands, WA, 6009, Australia.
** Corresponding author. Department of Ophthalmology & Visual Science, Eye & ENT Hospital, State Key Laboratory of Medical Neurobiology, Institutes of Brain Science, Shanghai Medical College, Fudan University, Shanghai, 200032, China.
E-mail addresses: xhsun@shmu.edu.cn (X. Sun), dyyu@lei.org.au (D.-Y. Yu).

http://dx.doi.org/10.1016/j.exer.2015.08.025
0014-4835/

interesting topic which needs to be further investigated to determine whether the iris is one of the major sources of oxygen and nutrients supplying the anterior segment. The disruption of this homeostasis could be an important pathogenic factor in glaucoma and cataract formation (Barbazetto et al., 2004; Holekamp et al., 2005; Shui et al., 2006).

Recently we have reported the distribution of porcine iris microvasculature and its endothelial cells using intra-luminal micro-perfusion, fixation, and silver staining (Yang et al., 2015). Our results showed that 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 relatively large capillaries. In addition, multiple orders of iris vessels with large input arteries and the spindle shape of the endothelia suggest a high blood inflow provides sufficient supply for material exchange, predominantly the delivery of oxygen, glucose, and other nutrients, between the blood stream and the iris stroma. This is assumed to be crucial for maintaining homeostasis in the ocular anterior segment, and in particular oxygen gradient homeostasis. It is also important to note that the anterior surface of iris does not form a continuous, impermeable sheet that overlies the anterior iris surface but allows aqueous humour to pass freely into the iris stroma (Freddo, 1996; Oyster, 2000). A modification of the stroma composed of a relatively dense meshwork of melanocytes and fibroblasts with associated collagen forms the anterior surface of the iris (Freddo, 1996; Hogan et al., 1971; Tousimis and Fine, 1959; Vrabec, 1952). Therefore, vascular endothelium in the iris could be a critical barrier for controlling homeostasis of the iris stroma and aqueous humour.

We hypothesized that endothelial cells were exposed to significant shear stress in the different locations and orders of the iris microvasculature, and that the cytoskeleton of the endothelium is central to meeting all these haemodynamic challenges. The actin cytoskeleton provides a strong and dynamic intracellular scaffold that organizes integral membrane proteins with the cell's interior, and responds to environmental cues to orchestrate appropriate cell shape (Prasain and Stevens, 2009). The roles and regulation of cytoskeletal components in the endothelium are critical for our understanding of endothelial function in both health and disease (Aird, 2007; Yu et al., 2014).

The function of the endothelium as a semi-permeable barrier can be broadly understood in terms of a balance between cell–cell and cell–matrix adhesion forces to maintain the integrity of the barrier (Curry and Adamson, 2010). Adherens, tight and gap junctions connect adjoining endothelial cells lining the vessel wall, playing pivotal roles in not only tissue integrity but also in vascular permeability, leukocyte extravasation and angiogenesis. There are complex interactions between signalling pathways that modify cell–cell adhesion. It has been reported that the adhesion protein VE-cadherin and the tight junction protein occludin, are both continuous around the periphery of venular endothelial cells in rat mesentery (Wallez and Huber, 2008). However, based on metabolic requirements, the presence and organization of inter-endothelial junctions vary in different organs, different tissues, and even in different vascular segments within the same tissue.

In this study, we examined the intracellular cytoskeleton and the distribution of key components of the adhesion mechanisms in junctions such as VE-cadherin and claudin-5 in different orders of iris microvessels.

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). Thirty seven eyes with sufficiently long temporal long posterior ciliary arteries (LPCA) and lightly pigmented irises were selected for this study (11 eyes for VE-cadherin staining, 10 eyes each for Phalloidin and claudin-5 staining, and another 6 eyes in total for double labelling). 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; Yang et al., 2015; Yu et al., 2003). Similar techniques were used in the present study and will be briefly described. Before perfusion, the nasal long posterior ciliary artery (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., 2010). Briefly, the pig eye was placed temporal side up in an eye holder, the temporal LPCA was cannulated using glass micropipettes with tip sizes of 270–300 μm, and secured in place by a 9-0 nylon suture.

Syringe pumps (model 22; Harvard Apparatus, South Natick, MA) were used to deliver an adjustable flow of perfusate and the perfusion pressure was continuously monitored through conventional transducers (Cobe, Arvada, CO), each connected to a bridge amplifier (model 5B38-02; Analog Devices, Norwood, MA) and recorded on a chart recorder (LR8100; Yokogawa, Tokyo, Japan). Additionally, a 25G needle connected to a pressure transducer was inserted into the anterior chamber to record the intraocular pressure (IOP). The flow rate was chosen to ensure that the iris vascular network could be perfused completely without IOP exceeding physiological level or inducing tissue swelling. Based on pilot experiments, we chose a baseline flow rate of 150 μL/min, and then adjusted this to 100 or 75 μL/min according to the IOP. The IOP was maintained close to the initial pressure at the perfusion beginning point (around 7–15 mmHg), and under 30 mmHg after the permeabilization step using Triton X-100. Oxygenated Ringer's solution with 1% bovine serum albumin was perfused for at least 30 min to flush out any residual blood. Then, solutions were perfused through in order with adjustments in protocols for phalloidin and antibody staining.

2.1. Perfusion labelling for actin microfilament

4% paraformaldehyde in 0.1 M phosphate buffer solution (PB) (30 min), 0.1% Triton-X 100 in 0.1 M PB (5 min), 0.1 M PB (45 min), the 850 μL mixture of Alexa Fluor 546 or 635 phalloidin (30U; A22283 or A34054, Invitrogen, Carlsbad, CA) and Hoechst (1.2 μg/ml bisBenzimide H 33258, Sigma–Aldrich, St. Louis, MO) in 0.1 M PB (90 min), and 0.1 M PB (40 min).

2.2. Intravascular VE-cadherin or claudin-5 immunohistochemistry

A protocol similar to that of phalloidin labelling was used, but 0.1% Triton-X 100 was prolonged to 8 min. This was followed by 0.1 M phosphate buffer wash (45 min), 10% donkey serum (1 h), and a goat anti-VE-cadherin primary antibody (1:50, 1 h; sc-6458, Santa Cruz Biotechnology, Santa Cruz, CA) or a rabbit anti-claudin-5 primary antibody (1:50, 1 h; SAB4200538, Sigma–Aldrich, St. Louis, MO). This was followed by a 0.1 M PB wash (45 min), and then secondary antibodies with Hoechst (H 33258, 1:1000, Sigma–Aldrich, St. Louis, MO) in 0.1 M PB was perfused (1 h) before the final wash with 0.1 M PB (45 min). Secondary antibodies used in this study were donkey anti-goat IgG (1:200; Alexa Fluor 488 or 555 A11055, Invitrogen, Carlsbad, CA or ab150130, Abcam, Cambridge, UK), and donkey anti-rabbit IgG (1:200; Alexa Fluor 488 A21206, Invitrogen, Carlsbad, CA).