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Correlation between the radial peripapillary capillaries and the retinal nerve fibre layer in the normal human retina

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ABSTRACT

This study aims to provide evidence of the importance of radial peripapillary capillaries (RPCs) by quantitative study of the relationship between the RPCs and retinal nerve fibre layer (RNFL) in normal human donor eyes. The retinal microvasculature in eleven normal human donor eyes was perfused, fixed and labelled after cannulation of the central retinal artery. The retinas were dissected and whole-mounted for confocal microscopy. Six study regions were taken radially from the edge of the optic disc. RPCs from the optic disc edge to a radial distance up to 2.5 mm were imaged and their diameters, inter-capillary distance and volume occupation measured. These were correlated with the study region as well as thickness of the RNFL. It was found that the pooled average diameter of the RPCs in the first 2.5 mm from the optic disk was 8.9 μm. Significant differences in capillary diameter were present in the six regions, with larger diameter RPCs in the superior, inferior and nasal regions, and significantly smaller diameter in the temporal region. RPCs in the arcuate fibre regions extend the furthest from the optic disc, maintained a close inter-capillary distance for a longer distance than other regions, and have the highest RPCs volume occupancy. The RPCs volume was generally correlated with RNFL thickness. In conclusion, a close correlation between RNFL and RPCs presence has been demonstrated which is supportive of their functional reliance/co-dependence. The significantly smaller temporal RPCs may be a result of the greater presence of RPCs in the two bordering arcuate fibre regions and therefore a richer availability of nutrients diffusing from these two regions.

1. Introduction

The radial peripapillary capillaries (RPCs) form a special vascular bed located in the retinal nerve fibre layer (RNFL) in the posterior pole (Henkind, 1967). Previous studies have provided some evidence to indicate that the RPCs may play an important role in the RNFL. The RPCs are only present in species with a typical macula, such as human and monkey; or species with equivalent specialised visual areas that are associated with a thicker RNFL in the posterior pole such as pig and cat (Henkind, 1967). Both histological and clinical studies have qualitatively described the RPCs to be the most prominent in the arcuate fibre region where the RNFL is also known to be the thickest (Henkind, 1967; Scoles et al., 2009). Numerous pathological changes such as Bjerrum scotoma, cotton wool spots, intra-retinal haemorrhage and ischaemic optic neuropathy all have in common a neural fibre defect that matches the distribution of RPCs and share common risk factors pertinent to ageing (Alterman and Henkind, 1968; Ashton, 1970; Kornzweig et al., 1968). The RPCs are postulated to be vulnerable to various pathological challenges and may be involved in the aetiology of eye diseases such as glaucoma and ischaemic retinal diseases, which are major causes of blindness in our community (Alterman and Henkind, 1968; Ashton, 1970; Daicker, 1975; Kornzweig et al., 1968). It is therefore critical to obtain solid quantitative data to confirm these postulations, that the RPCs has a role in maintaining the normal function of RNFL, and contributes to disease development in its dysfunction. However, there is currently no quantitative data to describe RPCs and in relation to the RNFL thickness in normal eyes. Nor is there quantitative descriptive index to detect for (progressive) change in ocular disease.

Abbreviations: RPCs, radial peripapillary capillaries; RNFL, retinal nerve fibre layer; Sup, superior; Temp, temporal; Inf, inferior; ST, supero-temporal; IT, infero-temporal; ODE, optic disk edge; RGC, retinal ganglion cells.

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In this study, we make use of the recently established technique of microperfusion, fixation and intravascular immunohistochemistry labelling, as well as whole mount retina and confocal imaging in human donor eye free from known ocular diseases to study the RPCs. We are able to perfuse label the RPCs in the intact retina and quantitatively study its distribution in correlation with RNFL thickness. RPCs endothelial cells have been labelled with endothelial specific markers.

2. Method & materials

The study was approved by the human research ethics committee at the University of Western Australia. All human tissue was handled according to the tenets of the Declaration of Helsinki.

2.1. Human donor eyes

All eyes were obtained from the Lions Eye Bank of Western Australia or Donate West, the West Australian Agency for Organ Donation. The corneal buttons were removed in all donor eyes by the eye bank staff for transplantation. None of the eyes used in the present study had a known history of eye disease.

11 eyes from 11 donors were studied. The donor's demographic data, cause of death, time to enucleation and post-mortem time to eye perfusion for each of the eyes are listed in Table 1. None of the eyes had record of ocular disease and are presumed to be free from ocular diseases.

2.2. Intravascular perfusion labelling

Details of the method of perfusion staining of retinal microvasculature have been published previously (Yu et al., 2010a, 2010b). Briefly, the central retinal artery was cannulated and residual blood washed out using oxygenated Ringer's solution with 1% bovine serum albumin. After 20 min of Ringer's wash, 4% paraformaldehyde in 0.1 M phosphate buffer was perfused for at least 20 min for light fixation. The perfusion protocol then followed depends on the structure to be labelled.

For labelling of microfilament (f-actin) and nuclei, a dilute detergent of 0.1% Triton-X-100 in 0.1 M phosphate buffer solution was perfused for 5–7 min to aid in the permeation of endothelial cell membranes. The detergent was then washed out by perfusion using 0.1 M phosphate buffer for 30 min prior to labelling using a mixture of phalloidin conjugated to Alexa Fluor 546 (30 U, Invitrogen A22283) and a nucleic acid probe of either YO-PRO-1 (6.6 μM; Invitrogen Y3603) or bis-benzimide H33258 (Sigma–Aldrich, B2261) for 2 h. Excess label were subsequently washed out by perfusing 0.1 M phosphate buffer through for at least 30 min. For labelling of VE-cadherin (1:50; sc-6458, Santa Cruz Biotechnology, Santa Cruz) and Claudin-5 (1:50, SAB4502981, Sigma–Aldrich, Saint Louis, USA), a blocking serum of 10% donkey serum in 0.1 M phosphate buffer was infused for 1 h prior to incubation with primary antibodies for 1 h. Residual primary antibody was washed out thoroughly for 30 min using 0.1 M phosphate buffer. The appropriate secondary antibody (1:200, A21205 or A11055, Invitrogen) was then perfused through for another hour together with nucleic acid label of either YO-PRO-1 or bis-benzimide H33258. Excess label were washed out by perfusing 0.1 M phosphate buffer through for at least 30 min. All the eyes were subsequently immersion fixed and the retina dissected and whole-mounted for confocal microscopy study. All retinas were used in quantitation of RPCs morphology as all markers used will label for various endothelial junction proteins and will have no effect on vessel diameter or volume measurements.

Seven of the eyes were also subsequently float-labelled (Yu et al., 2010b) for neurofilament heavy (1:400; N0142, Sigma) to confirm the position of the nerve fibre layer.

Table 1
Donor's details.

Eye IDs	Age	Sex	COD	Time to enucleation (h)	Time to cannulation (h)
A	15	M	Suicide	11	22.5
B	33	F	Suicide	5	7
C	39	M	Respiratory failure	15.5	20
D	53	F	Aortic rupture	7	15
E	57	F	Hypoxic brain injury	5	10
F	59	M	Myocardial infarct	19.5	21
G	60	F	Cancer	5	7.5
H	71	M	Abdominal aorta aneurysm	16	17
I	75	F	Subdural haematoma/ stoke	16	18
J	17	F	Suicide	20	21
K	19	M	Suicide	8	11

2.3. Regions of study

As the RPCs project radially from the edge of the optic discs and have previously been reported to be present amongst the arcuate fibres; six sectors were selected for quantitative analysis. These include the superior quadrant at 12 o'clock, temporal or nasal quadrant at 3 or 9 o'clock in the respective eye, the inferior quadrant at 6 o'clock and also the supero-temporal and infero-temporal regions. The six regions are illustrated in Fig. 1 and designated superior (Sup), temporal (Temp), nasal (Nasal), Inferior (Inf), supero-temporal (ST) and infero-temporal (IT) in the following text.

2.4. Confocal imaging

Confocal microscopy was carried out on the immunolabelled retinas within 2 weeks of flat mounting. Images were collected using two Nikon C1 machines equipped with a 3 laser (405 nm, 488 nm and 532 nm) or 4 laser (405 nm, 488 nm, 546 nm and 635 nm) board, coupled with the Nikon E800 or the fully motorised Nikon i90 microscopes and the EZ-C1 (v.3.20) software. All the images were collected using Plan Apo ×20 (NA 0.4) or ×40 (NA

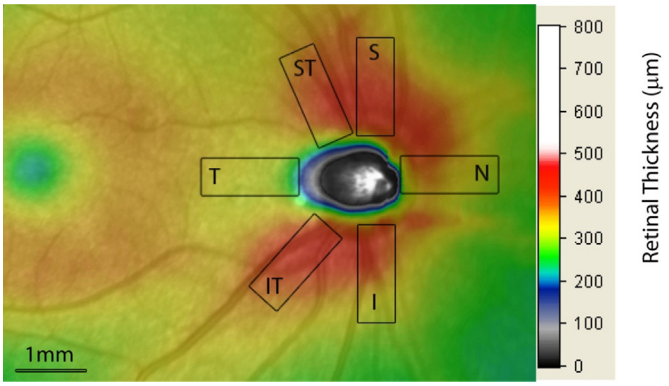

Fig. 1. Study regions superimposed on the OCT image of retinal thickness in the peripapillary region of a normal subject. The colour scale on the right corresponds with the colour coded retina for retinal thickness in microns. The six study regions are S – superior, N – nasal, I – inferior, IT – infero-temporal, T – temporal and ST – supero-temporal. The thickest regions of the retina have been included in the S, ST, IT and I study regions.