

Original Article

Effects of Travoprost on conjunctival vascular density and blood flow velocity

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Abstract: *Aim:* The conjunctiva hemodynamics changes lead to vascular dysfunction and pathophysiological changes. We aimed to evaluate the effect of Travoprost eye drops on conjunctival vascular density and blood flow velocity in healthy individuals. *Methods:* We recruited 16 healthy subjects treated with Travoprost eye drops (one of the prostaglandin analogs) and used functional slit lamp biomicroscopy (FSLB) to image the conjunctival noninvasive microvascular perfusion maps (nMPMs) and assess the hemodynamics. The high-speed camera sets at a high imaging rate of 60 frames per second and an up to 210× high magnification to achieve the hemodynamics. Fractal analysis of conjunctival nMPMs was analysed by customized software to measure both the blood flow velocity and the vascular density of the upper, lower, nasal, and temporal sides of each subject. We used the optical coherence tomography angiography (OCTA) to obtain the blood vessel images on both the left and right sides of each subject. The blood vessel density of both sides was measured and analyzed individually. *Results:* The vascular density of the lower side significantly increased after 6 hours of the Travoprost eye drops treatment ($p < 0.05$); however, the vascular density of the upper, nasal, and temporal sides, both the left and right sides were not in significant difference ($p > 0.05$). The FSLB and OCTA results were not significantly different in the conjunctival microvessel density ($p > 0.05$). The blood flow velocity increased slightly ($p > 0.05$). The blood flow velocity increased significantly after six hours and less obvious after 12 hours and was back to normal after 24 hours. The blood flow velocity of the lower side significantly increased with Travoprost eye drops treatment after six hours ($p < 0.05$); however, the vascular density of the upper, nasal, and temporal sides were not significantly different before the treatment and after six hours, 12 hours and 24 hours ($p > 0.05$). The blood vessel velocity between the left and right eyes was not significantly different ($p > 0.05$). *Conclusions:* In healthy individuals, Travoprost eye drops treatment could slow down the conjunctival blood flow velocity after a short period of time and has no significant effect on vascular density.

Keywords: Conjunctiva, vascular density, blood flow velocity, functional slit lamp biomicroscopy, optical coherence tomography angiography, travoprost

Introduction

The conjunctival microvessels are at the terminal of vascular beds above the sclera wrapped in the semipermeable membrane. The conjunctival microvessel changes in shape and hemodynamics would lead to microvascular dysfunction and a series of pathophysiological changes. Currently, the computer-assisted live microscopy [1-4] and adaptive slit lamp biomicroscope digital imaging [5-7] have been used as real-time noninvasive method for conjunctival blood flow measurement. These techniques could de-

tect specific vascular lesions on the conjunctiva. However, novel techniques are needed for detecting subtle changes in the microvascular network and quantifying the hemodynamics.

The functional slit lamp biomicroscopy (FSLB) was adapted from the traditional slit lamp microscope by attaching a digital camera with its cut image function greatly improved the slit lamp optical system magnification. FSLB has a higher spatial resolution and is ideal for conjunctival hemodynamics measurement. The high FSLB image magnification and recording

speed could be achieved without compromising image quality. FSLB allows researchers to record high-speed video recording for hundreds of blood vessels on the conjunctiva, noninvasive conjunctival microcirculation perfusion map, detailed microvascular network and microvascular circulation, and blood flow morphology and hemodynamics assessment through further image processing and analysis.

The optical coherence tomography angiography (OCTA) is a new noninvasive highspeed imaging system simultaneously showing the structural pathological changes and blood flow information, and can generate retina and choroidal layer structural data and blood flow information, compared to the invasive testing fluorescein angiography (FA) and indocyanine green angiography (ICGA) which needs intravenous dye and imaging time up to 30 minutes [8-11]. When compared to the traditional OCT, OCTA has a higher scanning speed to obtain a denser sampling volume without compromising the image quality and visual field decline [12-15]. We could comprehensively analyze the effect of Travoprost eye drops on conjunctival hemodynamics with the superficial vascular density information from the OCTA measurement and with the comparison of the FSLB results.

We aimed to investigate the hemodynamics of healthy individuals after receiving Travoprost eye drops by FSLB and OCTA measurements for exploring the effect of Travoprost eye drops on the hemodynamics of healthy conjunctiva.

Materials and methods

Patients

We recruited 16 healthy graduate students from the First Affiliated Hospital of Nanchang University and Zhongshan Ophthalmic Center, including 8 males and 8 females aged 21-25 years (mean age 22.6 years old), the intraocular pressure was 11-20 mmHg (averaged intraocular pressure is 16.8 mmHg), the vision was 0.8-2.0 (averaged vision is 1.0). All subjects had no ocular complications (such as retinopathy, eye infections, and etc.), ophthalmic disease surgery, various blood system diseases, cardiovascular and cerebrovascular diseases.

The study had been approved by the Scientific Ethical Committees in the two hospitals, and

the study was performed in accordance with the ethical principles in the Helsinki Declaration. Informed consent was obtained from all participants.

Methods

Travoprost eye drops treatment: Two drops of Travoprost eye drops were given to each eye of every participant during the same visit at 8:00, and then the conjunctiva hemodynamics on the upper, the lower, the nasal and temporal sides were measured at 8:00, 14:00, 20:00 and 8:00 the next day by the FSLB.

Microvessel blood flow measurement: The microvessel blood flow measurement method used in this study had been described in previous publication in detail by Jiang *et al.* [16]. The slit lamp was connected to the high-speed digital camera and a computer with customized software for analyzing the conjunctiva hemodynamics [5, 17]. The high-speed camera was set at a high imaging rate of 60 frames per second and an up to 210× high magnification to achieve the images with high spatial resolution and high magnification by the cutting function inherent in the camera. We obtained blood flow images with the size of 0.94×0.70 squared millimetre (mm²; 1.47 μm per pixel, up to 210× high magnification). During the image analysis process, the International Standards Organization was set to 400 and the shutter speed (SS) was set to 1/60 for determining the vessel wall, marking the blood vessel centerline, outlining the erythrocyte movement in the spatio-temporal image, measuring its slope, calculating the vessel diameter (D, μm) by the full width at the half maximum (FWHM), and measuring the arterial velocity (Va, millimetre per second: mm/s) and the venous velocity (Vs, mm/s). Four regions (**Figure 1A**) in each side were measured and the averaged results were calculated.

Microvessel density measurement: The microvessel density measurement method we used had been in detail described in previous publication by Jiang *et al.* [16]. After setting the image size to 5,184×3,456 pixels and the SS to 1/15 sec, 15.74×10.50 mm² temporal conjunctival vision field was captured by still image shooting mode with a green filter, then the noninvasive microvascular perfusion maps (nMPMs) was obtained. The microvascular images were segmented and the fractal analysis

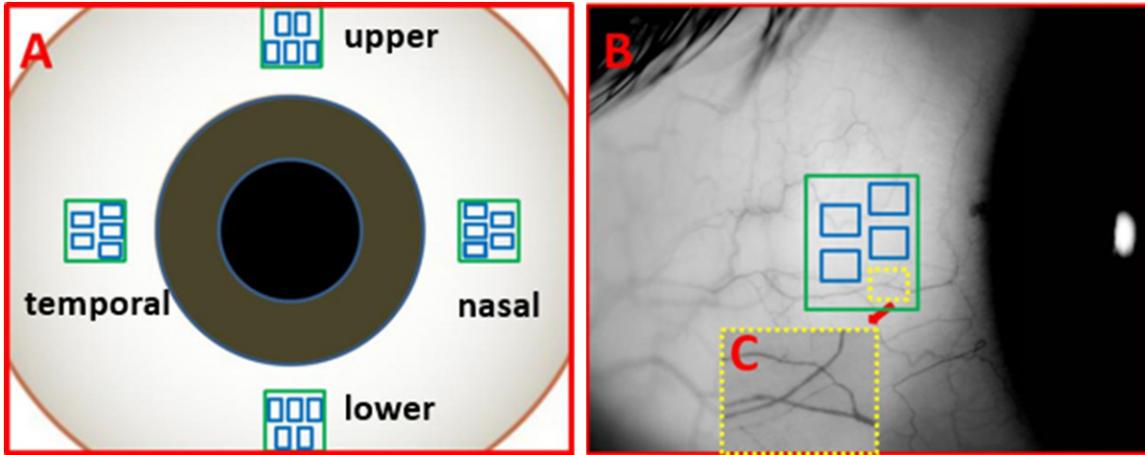


Figure 1. A. The regions of interest on the upper, lower, nasal and temporal sides of conjunctival microvessels (green squares) on the FSLB imaging. The regions of interest are set about 1 mm apart from the temporal lobe conjunctiva with five small different regions (blue rectangles). B. On the temporal side, we ensure more than 15 vessels are included in each small region and the image size is 640×480 in pixels. C. The vascularity on the temporal side imaging.

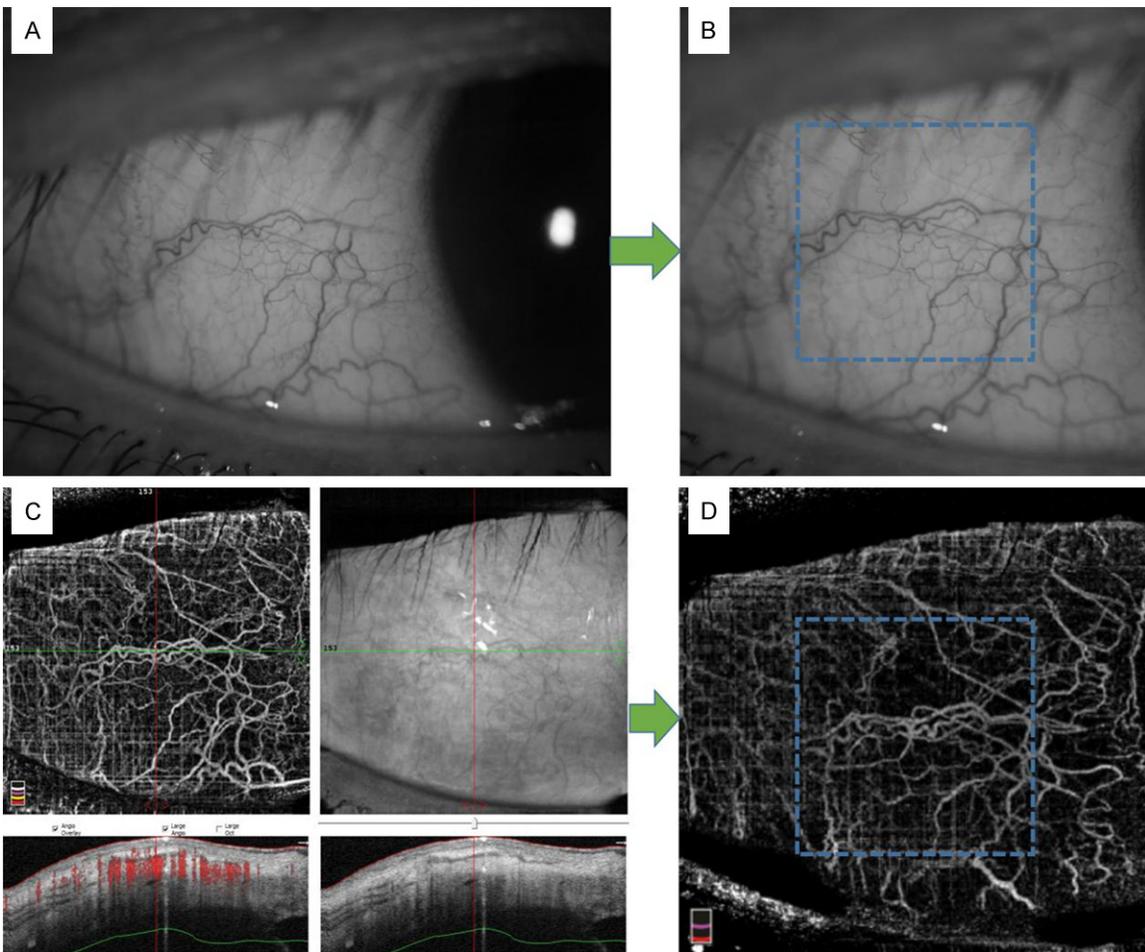


Figure 2. A and B. Fractal analysis of the conjunctival microvasculature. A. FSLB image of conjunctival microvessels. The limbus is imaged by FSLB about 1 mm from the temporal lobe conjunctiva. B. Image captured from video clip and the region of interest is set to 3×3 mm² (rectangle are in blue dashed line). C and D. OCTA images with a selective filter and a binary image. D. The region of interest is set to 3×3 mm² (rectangle are in blue dashed line).

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Table 1. Comparison of conjunctival microvessel density changes after treated with Travoprost eye drops

Part Time	Right Eye				Left Eye			
	0 h	6 h	12 h	24 h	0 h	6 h	12 h	24 h
Upper side	1.56±0.35	1.57±0.33	1.56±0.36	1.56±0.39	1.56±0.42	1.58±0.37	1.57±0.41	1.57±0.45
Lower side	1.58±0.43	1.63±0.41*	1.61±0.40	1.59±0.38	1.57±0.48	1.61±0.45*	1.59±0.43	1.58±0.42
Nasal side	1.61±0.42	1.63±0.41	1.62±0.38	1.62±0.44	1.62±0.47	1.63±0.39	1.63±0.44	1.62±0.38
Temporal side	1.59±0.36	1.61±0.39	1.60±0.37	1.59±0.42	1.60±0.39	1.62±0.41	1.61±0.35	1.60±0.41

h: hours; *: $p < 0.05$

was performed by the customized software [6] to obtain the vascular density and to analyze the nMPMs.

Vascular density by OTCA and image analysis:

The vascular density was measured by OTCA, and the parameters were set as previously in detail described by Spaide RF [15]. The axial resolution of the optical adapter lens was set to 5 μm , the beam width to 22 μm , the light source center to 840 nm, and scanning speed to 70,000 times per second which took three to four seconds to create a scanning cube. The lens adapter was set to two to four centimeters from the participant's corneal surface for preventing contact between adapter lens and glasses, the focal length was manually adjusted and focused until the image was clear. The images of right eyes from nasal and temporal sides were taken after adjusting.

The images were exported to ImageJ 1.38X (National Institutes of Health, Bethesda, MD) for further analysis [18]. The scanned area (**Figure 2C**) was identified by five consecutive rectangles (100 pixels; 850 μm) by approximately 4 mm along the circumference of the edge of the limbus conjunctivae. The blood vessel linear structure was highlighted by a selected filter to generate binary images for further processing. The following indexes were also evaluated: the number of vascular rings [19], vessel segments (previously defined as the part between two branch or end points) [19], fractal dimensions and the areas of each vascular ring passing through the closed area (mm^2) [20, 21]. A recognition system was used to evaluate the quality of scanned OTCA images according to the signal strength and image quality and to score the images to 0-4 points: point 0, can not distinguish vessels; point 1, poor quality of vascular images; point 2, good quality of vascular images; point 3, better qual-

ity of vascular images; point 4, excellent quality of vascular images.

Statistical analysis

Repeated measure ANOVA and post-hoc analysis was used for all indexes to compare before and after treatment, and SNK-Q test was applied for all indexes between the two groups of multiple comparisons in same area. $P < 0.05$ was considered significant. All data were analyzed by SPSS version 17 (IBM Inc, Armonk, NY).

Results

Conjunctival microvessel density by the FSLB measurement

A total of 16 healthy participants are enrolled in this study. The conjunctival microvessel density results measured by FSLB are shown in **Table 1**. The vascular density of the lower side significantly increased after 6 hours of the Travoprost eye drops treatment ($p < 0.05$). The microvessel density of the upper, lower, nasal and temporal conjunctiva was not different significantly ($p > 0.05$). There was no significant difference in the vascular density between the nasal and temporal parts at the same time point at 6 h, 12 h and 24 h after treated with Travoprost eye drops ($p > 0.05$). There was no significant difference in conjunctival microvessel density between the left and right eyes ($p > 0.05$).

Conjunctival microvessel density comparison between FSLB and OCTA measurements

The conjunctival microvessel density of right eyes comparison between FSLB and OCTA measurements are shown in **Table 2**. The comparison of microvessel density of the right eyes between FSLB and OCTA measurements was not significantly different ($p > 0.05$, **Table 2**).

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Table 2. Conjunctival microvessel density of the right eyes comparison between FSLB and OCTA measurements

Part	FSLB measurement				OCTA measurement			
	0 h	6 h	12 h	24 h	0 h	6 h	12 h	24 h
Nasal side	1.62±0.44	1.65±0.49	1.63±0.51	1.63±0.49	1.61±0.42	1.63±0.41	1.62±0.38	1.62±0.44
Temporal side	1.61±0.47	1.63±0.48	1.61±0.48	1.61±0.46	1.59±0.36	1.61±0.39	1.60±0.37	1.59±0.42

h: hours; *: $p < 0.05$

Table 3. Conjunctival arteriolar flow velocity measurements

Part	Right Eye				Left Eye			
	0 h	6 h	12 h	24 h	0 h	6 h	12 h	24 h
Upper side	0.45±0.18	0.47±0.19	0.46±0.16	0.45±0.17	0.46±0.22	0.48±0.19	0.47±0.21	0.46±0.21
Lower side	0.48±0.21	0.52±0.16*	0.49±0.23	0.49±0.22	0.49±0.19	0.53±0.17*	0.51±0.21	0.49±0.22
Nasal side	0.44±0.19	0.46±0.22	0.45±0.17	0.44±0.21	0.43±0.14	0.45±0.16	0.44±0.17	0.43±0.16
Temporal side	0.42±0.18	0.44±0.19	0.43±0.21	0.42±0.22	0.43±0.16	0.44±0.18	0.44±0.18	0.43±0.17

h: hours; *: $p < 0.05$

Table 4. Conjunctival venular flow velocity measurements

Part	Right Eye				Left Eye			
	0 h	6 h	12 h	24 h	0 h	6 h	12 h	24 h
Upper side	0.33±0.16	0.35±0.17	0.34±0.14	0.33±0.15	0.34±0.16	0.36±0.19	0.35±0.17	0.34±0.18
Lower side	0.31±0.22	0.35±0.24*	0.33±0.21	0.32±0.19	0.32±0.18	0.36±0.17*	0.33±0.19	0.32±0.21
Nasal side	0.30±0.19	0.32±0.21	0.32±0.18	0.30±0.17	0.31±0.19	0.33±0.17	0.32±0.21	0.31±0.18
Temporal side	0.31±0.15	0.33±0.18	0.32±0.16	0.31±0.17	0.32±0.16	0.34±0.17	0.33±0.18	0.32±0.19

h: hours; *: $p < 0.05$

Conjunctival arteriolar blood flow velocity measurements

Conjunctival arteriolar blood flow velocity measurement results of the upper, lower, nasal and temporal arterioles are shown in **Table 3**. The arteriolar blood flow velocity increased slightly ($p > 0.05$), the arteriolar blood flow velocity increased significantly after six hours and less obvious after 12 hours and had been back to normal after 24 hours. The arteriolar blood flow velocity of the lower side significantly increased after 6 hours with Travoprost eye drops treatment ($p < 0.05$), however, the arteriolar blood flow velocity of the upper, nasal, and temporal sides were not in significantly different before the treatment and in six, 12 and 24 hours after ($p > 0.05$). The arteriolar blood flow velocity between the left and right eyes was not significantly different ($p > 0.05$).

Conjunctival venular blood flow velocity measurements

The conjunctival venular blood flow velocity results shown in **Table 4** were similar to the

arteriolar blood flow velocity results. The venular blood flow velocity of the lower side significantly increased after 6 hours with Travoprost eye drops treatment ($p < 0.05$); however, results from other parts or differences between left and right eyes were not significantly different ($p > 0.05$).

Discussion

In this study, FSLB and OCTA were used to measure the blood flow velocity and blood vessel density of the subjects by applying the Travoprost eye drops to normal individuals. Both of them were found to increase the microvascular density of the binocular conjunctiva in a short time, but only had a slight effect on the blood flow rate. Thus, FSLB and OCTA could detect some subtle changes in the conjunctival microvessels and makes the quantitative analysis of the conjunctival microvessels morphology and hemodynamics possible.

Slit lamp microscope utilizes the reflective area on cornea or lens surface area for lesion detection and progression. It is the most popular

equipment, however not suitable for conjunctival microvascular system hemodynamics measurement. Though some parameters could be used in serious stage of disease, it is inconvenient for subtle vascular abnormalities and biomarkers in early stage of disease [7, 22, 23]. The FSLB is an adaptive method based on the traditional slit lamp microscope extensively used in the conjunctival microvascular system by connecting to a high-speed digital camera. The high-speed video recording function could generate the noninvasive ball conjunctival microcirculation blood perfusion map and make the microvascular network imaging range broader and clearer. FSLB may broaden the clinical application of classical optical devices [5-7] and reveal early signs of disease, and may provide more useful information for understanding disease etiology, disease progression and better patient care.

OCTA is a new technology with great potentials in clinical practice, for example, it could be used to evaluate the images of common eye diseases such as glaucoma, arterial and venous obstruction. OCTA images could generate more consistent capillary network with high signal-to-noise ratio and reduced motion artifact, based on the split-spectrum amplitude-decorrelation angiography algorithm [24]. OCTA technique could achieve vascular visualization by motion contrast imaging obtained by continuous B scans [15]. OCTA could generate high quality images within three to four seconds without intravenous dye or contrast agent injection [25]. The En-face function could obtain 3D image, which is convenient to observe pathological extent and details in the new blood vessels generation such as length, diameter and range [25]. There is a study suggesting OCTA has a higher vascularization than FSLB [26].

We carefully designed this study to explore the effect of Travoprost eye drops on the conjunctival blood vessel density and blood flow velocity in healthy participants, and we found no obvious change vascular density after the Travoprost eye drops treatment. The results could be explained by different distribution of arteriovenous according to the conjunctiva microcirculation characteristics. The blood flow velocity in the upper and lower sides was significantly greater the nasal and temporal sides might due to the effect of gravity. Moreover, the blood flow

velocity might also be affected by participants' hormone levels as well as room temperature; for example, the room temperature might be higher during the daytime than early morning or night. Travoprost is one of the prostaglandin analogs selectively binds to the prostaglandin F receptor with the side effects of conjunctival congestion and dry eye symptoms. The prostaglandin stimulation could cause elevated endothelial derived nitric oxide (NO) levels and increase blood vessel dilation and blood flow which may lead to conjunctival hemorrhage [27]. The NO synthase exists in the eye, even on the eye surface. Therefore, the microvascular blood flow velocity are partly caused by the prostaglandin stimulation, and may influence sensory nerves around the blood vessel walls and further increase blood flow in a short time period.

We found no obvious conjunctival microvascular density changes on both the nasal and the temporal sides in the right eye obtained by either FSLB or OCTA method. We then compared the results from FSLB and OCTA, and the results were consistent. We found the nasal side vascular density was greater than the temporal side vascular density by OCTA, which is consistent with Marcus Ang's study [28]. The greater nasal side vascular density may be due to patients long-term habits, and more pathological conditions in the nasal side. In this study, we could not consider the external factors such as contact lens [29], gene [30] and contact with eye drop containing thimerosal, neper gold, and chlorhexidine which may influence blood vessel structure in eyes. Further follow-up studies are needed to investigate the long-term effect of Travoprost eye drops on microvascular density as well as on glaucoma treatment. Parameters of FSLB and OCTA could be adjusted for patients' hemodynamic monitoring.

In this study, the blood flow velocity and blood vessel density of the subjects were measured, and the influence degree of prostaglandin was found to some extent. Compared with past measurement methods, the results were more accurate and detailed. It can also reflect the effect of Travoprost eye drops to a greater extent and quantify its effects. However, this experiment has some limitations. Because the sample size of the experiment was relatively small, it was not possible to determine whether

the effects of Travoprost eye drops on all healthy individual conjunctival vascular density and blood flow velocity in the short term had the same effect. At the same time, due to the follow-up time is short, we cannot be sure with the extension of time, Travoprost eye drops are for other impact on conjunctival blood flow velocity and the density of blood vessels, this needs us to late sample size and more lasting.

In conclusion, travoprost affects conjunctival vascular density and blood flow velocity detected by FSLB and OCTA. Follow-up studies are needed to investigate the long-term travoprost effects on eye hemodynamics.

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Disclosure of conflict of interest

None.

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