Effects of Pentoxifylline and Alprostadil on Ocular Hemodynamics in Healthy Humans

Guido T. Dorner,[1,2] Claudia Zawinka,[1] Hemma Resch,[1] Michael Wolzt,[1] Leopold Schmetterer,[1,3] and Gerhard Garhofer[1]

PURPOSE. Alprostadil, a prostaglandin (PG)E_1 analogue and pentoxifylline, an alkylxanthine derivate, have been shown to exert vasodilatory effects in several vascular beds. The purpose of the present study was to investigate the effect of PGE_1 and pentoxifylline on the ocular circulation.

METHODS. A placebo-controlled, double-masked, three-way, crossover study was performed in 15 healthy male subjects. Subjects received pentoxifylline (300 mg), PGE_1 (alprostadil 60 μg), or placebo intravenously over 2 hours on three trial days. Choroidal red blood cell flow was assessed with laser Doppler flowmetry and pulsatile choroidal blood flow with laser interferometric measurement of fundus pulsation amplitude (FPA). Retinal blood cell flow was calculated based on the measurements of maximum erythrocyte velocity in a retinal vein assessed with bidirectional laser Doppler velocimetry, and diameter measurements of retinal vessels were obtained with a retinal vessel analyzer.

RESULTS. Pentoxifylline increased FPA by 15.4% ± 1.1% ($P <$ 0.001 versus placebo and baseline). Alprostadil tended to increase FPA, but this effect did not reach the level of significance ($P = 0.07$ versus placebo). Choroidal blood flow as measured with laser Doppler flowmetry tended to increase during pentoxifylline and PGE_1 infusion by 8.9% ± 2.9% ($P = 0.062$) and 4.5% ± 6.2% ($P = 0.29$), respectively, but none of these effects was significant. The drugs under study had no effect on mean red blood cell velocity in retinal veins, on retinal vessel diameters, intraocular pressure, blood pressure, or pulse rate.

CONCLUSIONS. PGE_1 did not alter the parameters of retinal or choroidal circulation in healthy subjects. Pentoxifylline increased FPA, but did not change choroidal blood flow as measured with laser Doppler flowmetry and did not affect retinal blood flow parameters. Accordingly, neither pentoxifylline nor PGE_1 appears to be suitable to improve ocular blood flow in healthy subjects. Whether long-term treatment with alprostadil would improve choroidal blood flow in patients with vascular disease remains to be established. (*Invest Ophthalmol Vis Sci.* 2007;48:815–819) DOI:10.1167/iovs.06-0823

The discovery of multiple endothelium-derived substances—in particular, nitric oxide (NO), endothelins, prostaglandins (PG), and others—have markedly increased our understanding of local blood flow regulation.[1] Whereas the role of NO and endothelins in local blood flow regulation of the eye has been extensively studied,[1,2] knowledge about the effects of prostaglandins on ocular blood flow is still sparse. The paucity of knowledge can be at least partially attributed to the great variety of different PG subtypes and the heterogeneity of effects in this drug group, which includes vasodilation (PGI_2), vasoconstriction (PGH_2), and antithrombotic as well as platelet antiaggregatory properties.

Given that specific PG analogues have been identified that combine both a strong vasoactive and antithrombotic potential, PGs have been proposed as a potential therapeutic approach in patients with vascular disease. In particular, alprostadil, a vasoactive PGE_1 analogue, is widely used in the treatment of vascular diseases associated with impaired endothelial cell function.[3] Alprostadil has been demonstrated to exert direct and indirect vascular actions such as vasodilation and enhancement of blood viscosity.[3] In addition, alprostadil has fibrinolytic, antithrombotic, and platelet antiaggregatory properties.[4] Whereas alprostadil has been used in patients with critical limb ischemia for several years, its effect on the ocular vasculature has not been adequately studied so far, and only a few anecdotal reports are available.[5,6]

Pentoxifylline, an alkylxanthine derivative that has been used in different vascular conditions associated with ischemia, has also been proposed as a therapeutic option in ocular vascular disease, based on the potential of the drug to increase parameters of retinal and choroidal perfusion in healthy subjects and patients with retinal disease.[7–10] Accordingly, the purpose of the present study was to investigate the effects of alprostadil on choroidal and retinal perfusion and to compare them to the actions of pentoxifylline. To test the hypothesis that these two drugs are capable of increasing blood flow to the posterior pole of the eye, we performed a randomized, placebo-controlled, three-way, crossover study in healthy subjects and used an array of noninvasive technologies for the assessment of ocular perfusion parameters.

SUBJECTS AND METHODS

The protocol was approved by the Ethics Committee of the Vienna University School of Medicine and was conducted in accordance with the Declaration of Helsinki, including current revisions, and Good Clinical Practice guidelines. After written informed consent was obtained from all subjects, 15 healthy volunteers between 21 and 54 years of age (mean ± SD: 31.5 ± 9.3) were enrolled in the study. Each volunteer passed a screening examination that included medical history, a physical examination, 12-lead electrocardiogram, complete blood tests, urine drug screen, hepatitis B and C serologic tests and human immunodeficiency virus antibody tests. Subjects were asked to refrain from alcohol and caffeine for at least 12 hours before the study days. All subjects were studied with pupils dilated after instillation of tropicamide (Mydriaticum; Agepha, Vienna, Austria).

Study Protocol

In a double-masked, placebo-controlled, three-way, crossover design subjects were randomized to receive PGE_1 (alprostadil 60 μg diluted in

250 mL of physiologic saline solution; Prostavasin; Schwarz Pharma, Monheim, Germany), pentoxifylline (Trental; 300 mg diluted in 250 mL of physiologic saline solution; Aventis Pharma Deutschland GmbH, Frankfurt-am-Main, Germany), or placebo (250 mL physiologic saline solution) on three trial days. Each infusion period was 120 minutes. The washout between trial days was at least 7 days.

All treatments were administered into cubital veins by using an automatic device (IP 85-2; Sanitas, Salzburg, Austria). Noninvasive hemodynamic measurements were performed in a predetermined order at baseline and 1, 2, and 3 hours after the start of drug infusion.

Noninvasive Measurement of Systemic Hemodynamics

Blood pressure was measured noninvasively at 10-minute intervals on the upper arm by an automated oscillometric device (CMS patient monitor; Hewlett Packard, Palo Alto, CA). The pulse rate was monitored continuously with a finger photoplethysmographic device (CMS patient monitor; Hewlett Packard). The sensitivity of this equipment has been reported previously.[11] Mean arterial pressure (MAP) was calculated as diastolic blood pressure + ⅓(systolic blood pressure − diastolic blood pressure). Pulse pressure amplitude (PPA) was calculated as systolic blood pressure − diastolic blood pressure.

Fundus Pulsation Amplitude Measurements

Synchronous pulsations of the ocular fundus were assessed by laser interferometry in the subject's right eye with a method described in detail by Schmetterer et al.[12] Briefly, the eye is illuminated by the beam of a single-mode laser diode ($\lambda = 783$ nm) along the optical axis. The light is reflected at both the front side of the cornea and the retina. The two re-emitted waves produce interference fringes from which the distance changes between the cornea and retina during a cardiac cycle can be calculated. The maximum distance change is called fundus pulsation amplitude (FPA) and estimates the pulsatile choroidal blood flow.[13,14]

Choroidal Red Blood Cell Flow

Measurement of subfoveal choroidal red blood cell flow (RBC Flow$_{chor}$) was performed by laser Doppler flowmetry (LDF), according to the method of Riva et al.[15] In the present study, a commercially available fundus camera–based LDF systems was used (model 4000; Oculix, Sarl Arbaz, Switzerland). With this technique, the vascularized tissue is illuminated by coherent laser light, and scattering by moving red blood cells leads to a frequency shift in the scattered light. In contrast, static scatters in tissue do not change the light frequency, but lead to randomization of light directions impinging on red blood cells. This diffusion of light in vascularized tissue causes a broadening of the spectrum of scattered light, from which the choroidal blood flow can be calculated in relative units. In the present study, laser Doppler flowmetry was performed in the fovea to assess subfoveal choroidal red blood cell flow.

Retinal Red Blood Cell Velocity

Red blood cell velocities (RBC Vel$_{ret}$) were assessed using a bidirectional fundus camera–based laser Doppler velocimeter (LDV; model 4000; Oculix Sarl). The principle of red blood cell velocity measurement by LDV is based on the optical Doppler effect. Laser light, which is scattered by moving particles (e.g., erythrocytes) is shifted in frequency. The frequency shift is proportional to the red blood cell velocity in the retinal vessel. The maximum Doppler shift corresponds to the centerline erythrocyte velocity.[16] Using bidirectional laser Doppler velocimetry the absolute velocity in the retinal vessels can be obtained.[17] A main inferior or superior temporal retinal vein within 1 to 2 disc diameters was selected for measurements.

Retinal Vessel Diameter

The retina vessel analyzer (RVA; Imedos, Jena, Germany) is a commercially available system that comprises a fundus camera (FF 450; Carl Zeiss Meditec GmbH, Jena, Germany), a video camera, a real-time monitor, and a personal computer with analyzing software for accurate determination of retinal arterial and venous vessel diameters.[18] The fundus image is recorded by a video camera, digitized with a frame grabber, and displayed on a real-time monitor. Simultaneously, the signal is stored on a videotape, allowing off-line reanalyses of different vessel diameters. Each blood vessel has a specific transmittance profile due to the absorbent properties of hemoglobin. The software of the RVA calculates retinal vessel diameters with adaptive algorithms using these specific profiles.

To minimize variations in responses that may occur, depending on the fundus region, the same area along the blood vessel was selected in each subject. Vessel segments of inferior retinal branches as close as 1 to 2 disc diameters from the optic disc were used for measurements, allowing for determination of retinal vessel diameters with excellent reproducibility and sensitivity.[18]

Retinal Red Blood Cell Flow

Retinal red blood cell flow (RBC Flow$_{ret}$) through an individual major retinal vein was calculated from venous vessel diameters (VDv) and maximum blood velocity (Vel$_{max}$), which were taken from the same location at the studied vessel: Mean blood velocity (Vel$_{mean}$) in retinal veins was approximated as Vel$_{max}$/2. RBC Flow$_{ret}$ through a specific retinal vein can then be obtained as Vel$_{mean} \cdot \pi \cdot VDv^2/4$.

Intraocular Pressure and Ocular Perfusion Pressure

Measurements of intraocular pressure (IOP) were performed with Goldmann applanation tonometry on a slit lamp. Ocular perfusion pressure (OPP) was calculated as ⅔MAP − IOP.

Data Analysis

All statistical analyses were performed with commercial software (Statistica software package, rel. 4.5; StatSoft Inc., Tulsa, OK). The effects of alprostadil and pentoxifylline on outcome variables were assessed by a three-way, repeated-measures ANOVA model. Statistical significance was assessed as the interaction between time and treatment. Planned comparisons were used for post hoc testing. $P < 0.05$ was considered the level of significance. For data description, values are given as the mean ± SEM.

RESULTS

Systemic Hemodynamics and IOP

Baseline values of systemic and ocular hemodynamic measurements were comparable between all study days (Table 1). Pentoxifylline and alprostadil were well tolerated without any adverse events. Infusion of the drugs under study had no effect on MAP. A small decrease of pulse rate was observed in all groups over time, which was not different between treatments. IOP did not change during drug infusions (data not shown). In addition, no difference was observed in OPP and PPA between the three groups (Table 1). Neither OPP nor PPA was altered by any of the administered drugs (data not shown).

Alprostadil

Alprostadil tended to increase FPA by +3.9% ± 1.5% ($P = 0.054$ vs. placebo; Fig. 1) and RBC Flow$_{chor}$ by +4.5% ± 6.2%; $P = 0.29$; Fig. 1) after 2 hours of drug infusion. A tendency toward increased FPA was retained 1 hour after alprostadil infusion (+4.9% ± 2.0%). Arterial vessel diameter (VDa) (−1.2% ± 2.2%), VDv (−1.4% ± 0.8%), and RBC Vel$_{ret}$ (+4.9% ± 10.5%) were not changed by administration of alprostadil (Fig. 2).

TABLE 1. Baseline Hemodynamic Measurements on the Three Study Days

	Pentoxifylline	Alprostadil	Placebo
Mean blood pressure (mm Hg)	83 ± 1	81 ± 1	80 ± 1
Pulse rate (bpm)	69 ± 2	69 ± 2	72 ± 2
Fundus pulsation amplitude (μm)	4.29 ± 0.34	4.34 ± 0.35	4.33 ± 0.35
RBC flow$_{chor}$ (μL/min)	7.5 ± 0.4	7.6 ± 0.5	7.8 ± 0.4
RBC Vel$_{ret}$ (cm/s)	1.4 ± 0.2	1.3 ± 0.2	1.3 ± 0.1
VDv (μm)	157.5 ± 3.3	155.7 ± 4.6	156.0 ± 3.9
VDa (μm)	125.7 ± 4.3	128.4 ± 4.6	129.3 ± 4.7
IOP (mm Hg)	13.2 ± 0.7	13.1 ± 0.7	12.7 ± 0.6
Ocular perfusion pressure (mm Hg)	42 ± 5	41 ± 5	41 ± 6
Pulse pressure amplitude (mm Hg)	51 ± 10	51 ± 9	54 ± 8
RBC flow$_{ret}$ (μL/min)*	16.0 ± 3.3	15.8 ± 2.2	14.7 ± 2.2

Results are presented as means $\pm$ SEM ($n = 15$).
* Blood flow through the selected retinal vein under study and not total retinal blood flow.

Pentoxifylline

Pentoxifylline increased FPA by $+15.4\% \pm 1.1\%$ and $+12.4\% \pm 1.5\%$ (both $P < 0.001$ vs. baseline and placebo; Fig. 1) 1 and 2 hours after the start of infusion, respectively, and tended to increase RBC Flow$_{chor}$ by $+8.9\% \pm 2.9\%$ ($P = 0.062$ vs. placebo; Fig. 1). In contrast, placebo infusion had no effect on FPA ($-0.8\% \pm 1.2\%$) or RBC Flow$_{chor}$ ($-1.4\% \pm 5.1\%$). Retinal VDa ($+0.2\% \pm 1.7\%$) and VDv ($+0.4\% \pm 2.1\%$) were not altered during or after pentoxifylline infusion (Fig. 2). Pentoxifylline had no effect on RBC Vel$_{ret}$ ($-1.8\% \pm 15.8\%$, Fig. 2).
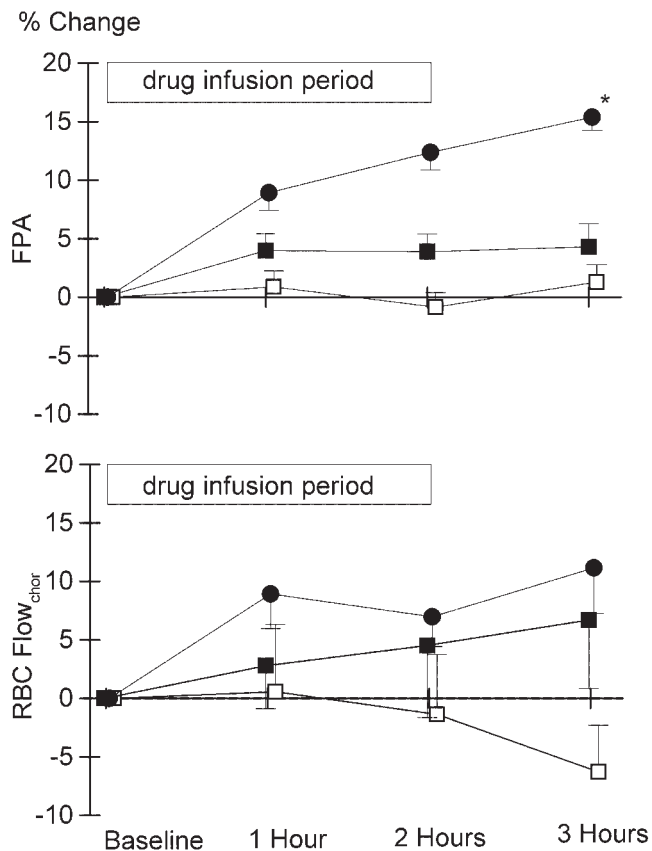

FIGURE 1. Effect of pentoxifylline (●), alprostadil (■), and placebo (□) on FPA and subfoveal RBC Flow$_{chor}$. *Horizontal bars*: drug infusion. Data are presented as the means $\pm$ SEM percentage change relative to baseline ($n = 15$). *$P < 0.001$ versus baseline and placebo.

DISCUSSION

PGE$_1$ is a naturally occurring PG, originally introduced as a therapeutic agent because of its potent direct vasodilator actions. Several experiments indicate a beneficial effect of intravenously administered PGE$_1$ in patients with chronic vascular disease (i.e., patients intermittent claudication or critical leg ischemia).[3,19] It has been hypothesized that these clinical effects of PGE$_1$ can be attributed to a direct potential vasodilatory effect of PGE$_1$ causing an increase in local tissue perfusion.

However, data on the blood flow effects of PGE$_1$ in the vascular bed of the eye are sparse. Early studies (1978) revealed an acute vasodilatory effect of PGE$_1$ when injected close to retinal arteries by iontophoresis.[20] Experimental evidence gained from a study treating patients with intermittent claudication over 21 weeks with PGE$_1$, indicated an increased flow velocity of the ophthalmic artery and the central retinal artery.[3] However, because increased blood speed does not necessarily reflect increased blood flow and because the study was not placebo controlled or masked for treatment, these results have to be interpreted with caution and do not necessarily support an ocular vasodilator effect. Data from animal experiments in cats indicate that intravenous administration of PGE$_1$ does not alter ocular blood flow, whereas a liposomal formulation of PGE$_1$ was found to induce a pronounced increase in optic nerve head blood flow.[21]

The results of our study indicate that intravenously administered PGE$_1$ has no acute effect on retinal or choroidal blood flow in healthy subjects. The experimental design of our study, however, differs significantly from the studies just mentioned. First, most of the reports demonstrating a beneficial effect of PGE$_1$ were performed in patients with endothelial dysfunction and may indicate that PGE$_1$ improves perfusion particularly in patients with compromised endothelial function. Therefore, one has to consider that the effects of PGE$_1$ measured in the present study reflect the situation in a vascular bed with an intact endothelium and could be different in patients with impaired ocular blood flow, altered perfusion pressure, decreased blood flow autoregulation capacity or endothelial dysfunction as observed for example in diabetes.[22]

Second, we were interested in whether alprostadil exerts an acute blood flow effect on the ocular circulation, whereas the latter reports investigated a long-term treatment with PGs over a period of 2 to 3 weeks. It has been shown that long-term administration of PGE$_1$ leads to an increase of VEGF and eNOS, which may in turn be beneficial for patients with endothelial damage.[23] In addition, it has been reported that prostaglandin infusion induces beneficial changes in soluble adhesion molecule plasma concentrations and intercellular adhesion molecules in patients with intermittent claudication.[24]
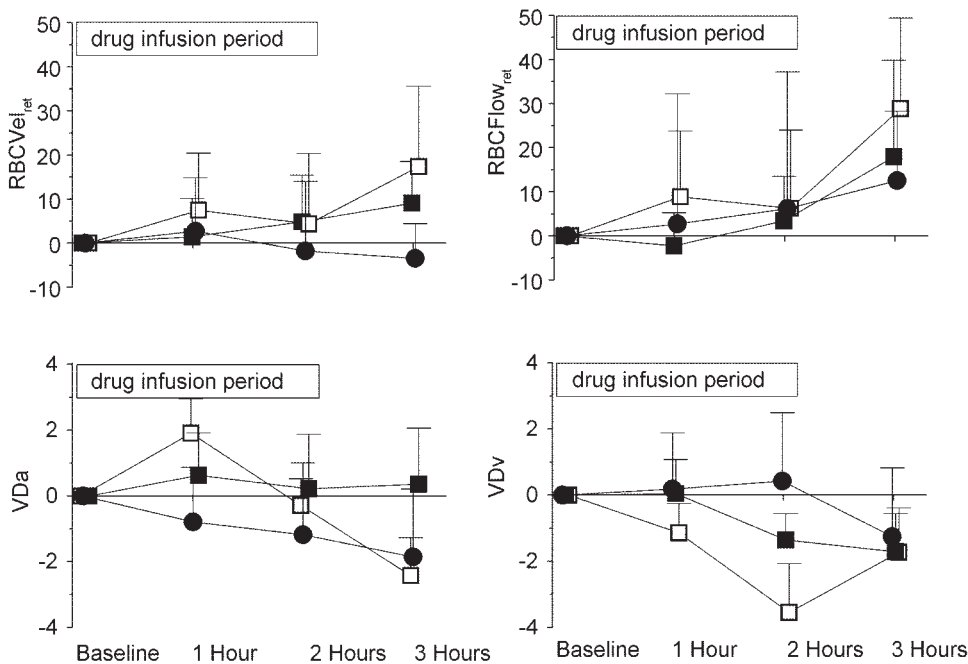
FIGURE 2. Effect of pentoxifylline (●), alprostadil (■), and placebo (□) on RBC Vel$_{ret}$, VDa, and VDv and calculated RBC Flow$_{ret}$ in a major retinal vein. *Horizontal bars:* drug infusion. Data are presented as the means ± SEM percentage of change relative to baseline ($n = 15$).

Pentoxifylline was chosen as a comparator based on own previous results: Equivalent intravenous doses had an equipotent effect on ocular fundus pulsation amplitude in different regions of the macula and the optic disc in healthy volunteers.[7] In patients with age-related macular degeneration, a 3-month oral administration of 300 mg pentoxifylline three times daily increased pulsatile choroidal blood flow by approximately 28%.[8] The data of the present study confirm the effect of intravenously administered pentoxifylline on pulsatile choroidal blood flow, as shown in previous studies,[7,8] whereas subfoveal choroidal blood flow as measured with laser Doppler flowmetry showed only a tendency to increase, but failed to reach the level of significance.

How can this difference be explained? It has to be to considered that, despite all efforts, no gold standard exists for the measurement of choroidal blood flow and that both laser Doppler flowmetry and laser interferometry have methodological limitations. Because laser interferometry assesses the pulsatile component of blood flow only, this technique is based on the assumption that changes in the pulsatile component of choroidal blood flow are proportional to changes in total choroidal perfusion. This hampers the interpretation of the results, because it is still a matter of controversy how much of choroidal blood flow is pulsatile.[25,26] These considerations regarding the pulsatile component of choroidal blood flow limit especially the conclusion in cross-sectional studies, but may also have an impact in longitudinal studies. In particular, a change in the PPA may considerably alter the ratio between pulsatile and nonpulsatile flow without any changes in choroidal blood flow.[14]

Based on the results of the present study it is difficult to answer the question of whether pentoxifylline may have changed flow pulsatility in the choroid. An increase in the ratio of pulsatile to nonpulsatile flow appears unlikely, because pentoxifylline did not induce any change in the blood pressure profile and induces vasodilation rather than vasoconstriction. With vasodilatation one would rather assume a shift from pulsatile to nonpulsatile blood flow. Accordingly, in such cases FPA may rather underestimate the effect on total choroidal blood flow.

In contrast to laser interferometry, LDF reflects total red blood cell flux. Thus, a more likely reason for the difference between results obtained by LDF and laser interferometry is related to the fact that the latter method assesses corpuscular erythrocyte flux only. In particular, the signal of the laser Doppler device is gained by the density and velocity of moving red blood cells. What is usually referred to as "choroidal perfusion" is calculated as the product of local velocity and concentration of moving blood cells. Hence, changes in blood viscosity[7] or local hematocrit in the subfoveal choroidal region during pentoxifylline may be at least partially responsible for the differences in choroidal blood flow estimates. In terms of treatment of ocular ischemic disorders one has to consider, however, that the oxygen transport capacity is dependent on red blood cell flux rather than on volumetric flow. Accordingly, our LDF data may indicate that that neither pentoxifylline nor alprostadil are capable of increasing oxygen delivery to the eye, even if the former improves volumetric flow as indicated by our FPA measurements. In addition, one has to mention that, because there is a lack of retinal vessels in the fovea, the system only allows for the subfoveal measurement of choroidal blood flow,[15] whereas the FPA most likely reflects the pulsation of a much wider area of the fundus.

It has been shown that laser Doppler flowmetry, also under best conditions has a worse reproducibility than laser interferometry.[27] Thus, the sample size calculation for the present study was based on the reproducibility data of LDF as published recently.[27] The 15 patients included in the present study allowed us to detect a difference between groups of 10% (two-sided 5% significance level; power of 0.8).

In addition, the present study was designed to investigate the effects of pentoxifylline and alprostadil on retinal blood flow. It has recently been reported that intravenous administration of pentoxifylline increases retinal capillary blood flow, as measured with scanning laser Doppler flowmetry (SLDF).[28] Further evidence demonstrated an increase in capillary white blood cell velocity assessed with the blue-field entoptic phenomenon.[9,10] In contrast, no effect of pentoxifylline on retinal blood flow was found by Kruger et al.[8], using SLDF, in patients with age-related maculopathy. The differences in these findings may be explained at least partially by the different techniques used. Both the blue-field phenomenon and the SLDF give an estimate of retinal capillary blood flow, whereas the technique used in the present study is based on measurements in major

retinal vessels. In addition, because of the specific technical properties of the SLDF system, it cannot be fully excluded that SLDF measurements are influenced by choroidal perfusion, an effect that could also contribute to the contradicting findings.[29,30]

In summary, our data indicate that pentoxifylline, but not alprostadil induces an acute increase in pulsatile blood flow in the choroid of healthy subjects, whereas none of the drugs altered red blood cell flow in the retina or choroid. Whether alprostadil can improve retinal or choroidal function in ischemic vascular diseases of the eye must be determined in future studies.

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