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Interaction between leukocytes and erythrocytes in the human retina: Effects of pentoxifylline on hyperoxia-induced vasoconstriction during increased neutrophil counts

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ABSTRACT

Purpose: Pentoxifylline, a nonselective phosphodiesterase inhibitor, shows vasodilator effects in certain vascular beds and reduces blood viscosity. We have previously shown that under states of vasoconstriction an interaction between circulating erythrocytes and leukocytes may play a role in the control of blood flow. The reason for this observation is not entirely clear but may be related to a mechanical interaction between red and white blood cells. In the present study we hypothesized that pentoxifylline may alter this interaction during oxygen-induced vasoconstriction.

Methods: 24 healthy male subjects participated in this double masked, randomized, placebo-controlled 2 way cross over trail. In order to increase white blood cell count (WBC) count, 300 μg of G-CSF was administered intravenously. Vasoconstriction of retinal vessels was induced by oxygen inhalation. 400 mg of pentoxifylline or placebo was infused at two different study days. White blood cell flux was assessed with the blue-field entoptic technique. Vessel calibers were measured with a dynamic vessel analyzer (DVA) and red blood cell velocity (RBCV) was determined with laser Doppler velocimetry (LDV). Retinal blood flow was calculated based on retinal vessel diameters and RBCV.

Results: Administration of G-CSF induced a significant increase in WBC, both in the placebo and the pentoxifylline group ($p < 0.01$ for both groups). Retinal vessel diameter, RBCV, calculated retinal blood flow and white blood cell flow were not altered by administration of pentoxifylline. Hyperoxia induced a pronounced decrease in retinal blood flow parameters. No difference was observed between groups during oxygen breathing in vessel diameters ($p = 0.54$), RBCV ($p = 0.34$), calculated retinal blood flow ($p = 0.3$) and white blood cell flow ($p = 0.26$).

Conclusion: Our data indicate that short time administration of pentoxifylline does not alter the oxygen-induced effect on ocular blood flow parameters during leukocytosis. Whether long-term treatment could improve retinal blood flow under states of vasoconstriction remains to be investigated.

Introduction

The complex regulation of ocular blood flow assures sufficient blood supply to the eye and consequently its proper function. However, this system is exposed to a lot of influences. Amongst others ocular blood flow can be altered via changes of the vessel diameter, perfusion pressure, or changes of blood rheology. For a long time erythrocytes were the focus of research (Schmetterer and Garhofer, 2007), as they outnumber other cellular components present in the blood and as they play a major role in oxygen transportation. Yet, epidemiological studies have shown that WBC could be a predictor for cardiovascular and future thrombotic events (Engler et al., 1983; La Celle, 1986; Schlant et al., 1982; Thompson et al., 1989).

Although the reason for this observation is still not fully understood, it has been hypothesized that a high number of WBCs may compromise blood flow by clogging vessels, particularly in the microcirculation. This hypothesis is also supported by the observation that WBCs show worse deformability (Braide et al., 1984) compared to erythrocytes. Together with the larger size of WBCs this may lead to "train" formation of the blood cells (Thompson et al., 1989) in the post-capillary venules and in turn to a reduction in blood flow, especially in the microcirculation.

Unfortunately, the effect of increased WBC count on blood flow regulation is difficult to investigate. We have shown in recent experiments that intravenous administration of granulocyte stimulating

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factor (G-CSF) is a suitable method to artificially increase WBC count in healthy human subjects and can serve as a human in-vivo model to study increased WBCs (Fuchsjager-Mayrl et al., 2002). Additional experiments showed that a G-CSF induced increase of WBCs can indeed alter retinal blood flow, especially under states of vasoconstriction. In particular, our results show that G-CSF alters blood flow regulation after oxygen or endothelin-1 induced vasoconstriction, indicating that increased WBC count leads to an impairment of blood flow when vessels are constricted (Lasta et al., 2012; Told et al., 2012).

Pentoxifylline (PTX) is a competitive nonselective phosphodiesterase inhibitor originally introduced for the treatment of peripheral circulatory disorders such as intermittent claudication. Pentoxifylline has also been widely used in ophthalmology for the treatment of ischemic eye disease (Demir et al., 2003; Park et al., 2007). Its drug effect has mainly been attributed to the fact that pentoxifylline improves red blood cell deformability and reduces blood viscosity (Muravyov et al., 2011). In the current study we hypothesized that pentoxifylline can antagonize the effect of increased WBC count on blood flow leading to improved retinal blood flow parameters in states of vasoconstriction. For this purpose, pentoxifylline or placebo was administered on two different study days in conditions of leukocytosis during oxygen-induced vasoconstriction.

Materials and methods

This study was conducted adhering to the tenets of the Declaration of Helsinki and the guidelines of Good Clinical Practice. The Ethics Committee of the Medical University of Vienna gave a positive vote for this study.

Subjects

After obtaining informed consent, 24 healthy male subjects aged between 19 and 35 years were scheduled for a prestudy screening visit during a 4 weeks period preceding the first study day. During the screening visit a physical examination was done, medical history was recorded, blood pressure and heart rate (supine and standing) were measured and 12-lead electrocardiogram was performed. In addition, blood samples were drawn to assess clinical chemistry (sodium, potassium, creatinine, alanine transaminase (GPT), gamma-glutamyltransferase (γ-GT), total bilirubin, total protein), the hematological status (hemoglobin, hematocrit, red blood cells (RBC), mean corpuscular hemoglobin (MCH), white blood cells (WBC), platelet count, activated partial thromboplastin time (aPTT), thrombin time). Furthermore a hepatitis B, C screening and HIV serology was performed. Subjects were only included if they had normal findings and blood parameters within normal limits.

Finally, an ophthalmic examination was performed. Ophthalmological inclusion criteria were normal ophthalmological findings, an ametropia less than 3 diopters, anisometropia less than 1 diopters and an intraocular pressure of 20 mm Hg or less.

Exclusion criteria were regular use of medication or alcohol abuse, smoking, a history of hypersensitivity to any of the drugs used or a history of a condition interfering with the distribution, metabolism or excretion of a study drug. Subjects were also excluded if they participated in a trial three weeks previous to the first study day.

Study design

The study design chosen was randomized, placebo-controlled, double masked two-way cross over.

Description of study days

Subjects had to abstain from alcohol and beverages containing caffeine for at least 12 h. A detailed time schedule of the study day is given in Fig. 1. Subjects were studied after an overnight fast and a 20 min resting period. Thereafter baseline measurements (M1) comprising the blue field entoptic technique, the dynamic vessel analyzer (DVA) and laser Doppler velocimetry (LDV) were performed, each taking about three to five minutes. This was followed by inhalation of 100% oxygen for 20 min and measurements were repeated during oxygen inhalation (M2). 3.5 h following G-CSF infusion (Granocyte, "Roche", Novartis, Basel, Switzerland, 300 μg single intravenous bolus infusion, (Fuchsjager-Mayrl et al., 2002)) blue-field entoptic technique, LDV and DVA measurements were repeated (M3). Finally pentoxifylline (Pentomer®, Merckle GmbH, Blaubeuren, FRG, Germany, intravenous infusion of 400 mg over 90 min, (Schmetterer et al., 1996)) or placebo (physiological saline solution) was administered and O2 inhalation was restarted. Again measurements were repeated during both PTX/Placebo (M4) and oxygen (M5) administration. Venous blood samples were drawn at baseline and after pentoxifylline application in order to detect white blood cell count (WBC).

Measurements

Blood pressure and pulse rate

An automated oscillometric device (HP-CMS monitor; Hewlett Packard, Palo Alto, CA, (Wolzt et al., 1995)) was used to measure systolic, diastolic and mean blood pressures at the upper arm. Pulse rate and oxygen saturation were assessed with a finger pulse-oxymetric device, attached to the monitor.

Intraocular pressure (IOP)

IOP was measured with an applanation-tonometer (Perkins MK2, Clement Clarke, Edinburgh, United Kingdom).

Oxygen breathing

100% oxygen for human use was delivered to the subjects by means of an airtight oxygen mask covering nose and mouth. The attached air tubes comprise an oxygen reservoir to ensure continuous oxygenation of 100%.

Blue-field entoptic technique

The commercially available system (Blue-field Simulator, Oculix Sarl, Arbaz, Switzerland) uses the optical blue field entoptic phenomenon in order to investigate the leukocyte dynamics in retinal perifoveal vessels (Riva and Petrig, 1980). The phenomenon is best observable at 430 nm of wavelength and shows many tiny corpuscles around an area of the center of the fovea, which result from different absorption spectra of red and white blood cells. Subjects were asked to match their own entoptic observation with the presented computer simulation by number (WBCD) and flow velocity (WBCV) in five consecutive runs. Only results varying less than 15% were considered accurate (Riva and Petrig, 1980).

Dynamic vessel analyzer (DVA)

By the means of a fundus camera (Zeiss FF 450, Jena, Germany), a video camera and a personal computer with special analyzing software, the commercially available DVA (Imedos, Jena, Germany) permits assessment of retinal vessel diameters (Blum et al., 1999; Garhofer et al., 2010). Fundus illumination was roughly 220 μW cm^2 and diameter detection in the video analysis had a time resolution of 25 readings per second. The system provides excellent reproducibility and sensitivity (Garhofer et al., 2010; Polak et al., 2000). A typical measurement before and during oxygen induced vasoconstriction is shown in Fig. 2.