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Effects of increased white blood cell count on endothelin-induced vasoconstriction in healthy subjects

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ABSTRACT

It is known that administration of granulocyte-colony stimulating factor is followed by an increase of white blood cell count. There is evidence from other vascular beds that an increase in white blood cell count impairs blood flow regulation especially in the microcirculation. Whether this also holds true for the ocular circulation is unknown. In the following study we investigated whether an increase in white blood cell count alters the endothelin-1 induced vasoconstriction in humans. Neither granulocyte-colony stimulating factor nor endothelin-1 had any consistent effect on blood pressure, pulse rate or intraocular pressure. Administration of granulocyte-colony stimulating factor induced a pronounced increase in retinal white blood cell density ($p < 0.01$). Administration of endothelin-1 decreased choroidal ($p < 0.01$) and retinal blood flow ($p < 0.01$). The change in choroidal blood flow in response to endothelin-1 was not altered by pre-treatment with granulocyte-colony stimulating factor. By contrast, the decrease in retinal blood flow was more pronounced during an increase in white blood cell count ($p = 0.02$) when compared to placebo. Our data indicates that during pronounced vasoconstriction, as induced by administration of endothelin-1, vascular regulation can be altered by the number of circulating white blood cells. Whether this effect is caused by an interaction of red and white blood cells in the microcirculation or a yet unknown mechanism needs further investigation.

1. Introduction

The regulation of ocular blood flow is a highly complex phenomenon assuring adequate perfusion of the organ and in turn, the proper function of the eye (Schmidl et al., 2010). Thus, it does not come as a surprise that even small alterations in the regulation of vascular tone or changes in the composition of the blood itself may result in compromised blood flow. For a long time the focus of research has been on erythrocytes because of their unique rheological properties and their role in oxygen transportation. However, epidemiological studies have stimulated interest in the role of the white blood cells (WBC) on blood flow regulation (Schlant et al., 1982).

In contrast to erythrocytes, WBCs form less than 1% of the total number of blood cells. Nevertheless, evidence has been provided that WBC count is a consistent predictor for both future thrombotic and cardiovascular events (Schlant et al., 1982). The exact reason for this effect is still a matter of research. It has, however, been hypothesized that an increase in WBC count may lead to accumulation and plugging of WBCs in the microcirculation and in turn to an increased risk for thrombotic events. This seems reasonable because WBCs, despite their low concentration, are larger in size and are less deformable compared to erythrocytes (Braide et al., 1984). Along this line of thought it has been shown that an increased WBC count leads to increased vascular resistance in isolated organs (Sutton and Schmid-Schonbein, 1992).

Unfortunately, in-vivo testing of the hypothesis that increased WBC count alters blood flow regulation in humans is difficult. We have recently shown that administration of granulocyte-stimulating factor (G-CSF) is an adequate model to increase WBC count

Abbreviations: G-CSF, Granulocyte colony stimulating factor; ET-1, Endothelin-1; IOP, Intraocular pressure; WBCD, White blood cell density; WBCV, White blood cell velocity; WBCF, White blood cell flux; DVA, Dynamic vessel analyzer; LDV, Laser Doppler velocimetry; LDF, Laser Doppler flowmetry; RBC, Red blood cells; WBC, White blood cell; Vel, Velocity; Vol, Volume; SD, Standard deviation.

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in healthy subjects (Fuchsjager-Mayrl et al., 2002). In order to investigate to which degree an increased WBC count can influence ocular blood flow and its regulation, we set out to investigate ocular blood flow during an increased WBCs count. This was done in both the absence and presence of endothelin-1 induced vasoconstriction. Given that the effects of WBCs on vascular resistance have been mainly attributed to their mechanical properties, (Sutton and Schmid-Schonbein, 1992) one could hypothesize that a blood flow effect, if there is any, will crucially depend on the capillary lumen dimensions and, thus, be stronger under states of vasoconstriction. Endothelin-1, as used in the current study, has been shown in a variety of human studies to be a strong vasoconstrictor and reduces retinal, optic nerve head and chorodial blood flow (Fuchsjager-Mayrl et al., 2003; Polak et al., 2001; Schmetterer et al., 1997). The present study tested the hypothesis that an increase in WBC count leads to a change in ocular blood flow regulation during states of vasoconstriction.

2. Materials and methods

2.1. Subjects

The present study was performed adhering to the tenets of the Declaration of Helsinki and the guidelines of Good Clinical Practice. After approval of the study protocol by the Ethics Committee of the Medical University of Vienna and after obtaining informed consent, 24 healthy male subjects, aged between 19 and 35 years were included.

All subjects underwent a prestudy screening during 4 weeks preceding the first study day, comprising medical history, physical examination, blood pressure and heart rate (supine and standing), 12 lead electrocardiogram, complete blood count, blood chemistry (sodium, potassium, creatinine, uric acid, glucose, cholesterole, triglycerides, alanine aminotransferase, aspartate transcarbamylase, γ-glutamyltransferase, alkaline phosphatase, total bilirubin, total protein levels), hematological status (hemoglobin, hematocrit, red blood cell count, mean corpuscular hemoglobin, white blood cell count, platelet count, activated partial thromboplastin time, thrombin time), Hepatitis B, C and HIV serology, urinalysis and ophthalmic examination. Subjects were excluded if any abnormality occurred during screening, unless judged clinically irrelevant by the investigators.

In addition, an ophthalmological examination was performed and only subjects with no ophthalmological pathologies were included. Further exclusion criteria were ametropia of more than 3 diopters and regular smoking.

2.2. Study design

Subjects were asked to abstain from alcohol and beverages containing caffeine for at least 12 h and were studied after an overnight fast. The study was performed in a randomized, placebo-controlled, double masked, two-way cross over design (Fig. 1).

2.3. Description of study days

On subjects' arrival a 20 min resting period was scheduled. Prior to intravenous infusion of 300 μg G-CSF (Granocyte®; Rohne-Poulenc Rorer Pharmazeutika, Vienna, Austria) (Fuchsjager-Mayrl et al., 2002) or placebo, baseline measurements with the blue-field entopic technique, laser Doppler flowmetry, laser Doppler velocimetry and the dynamic vessel analyzer were performed. In addition, IOP was measured and a venous blood sample was drawn to measure baseline WBC count.

After G-CSF/placebo infusion, a break of 8 h was scheduled. Then, WBC count was determined again by taking a venous blood sample. Thereafter, ET-1 (Clinalfa AG, Läufelfingen, Switzerland, 5 ng/kg/min) was administered intravenously for 30 min. The dose of 5 ng/kg/min was chosen based on previous experiments performed in our laboratory, indicating that at this dose induces a significant decrease in retinal and choroidal blood flow (Polak et al., 2003; Schmetterer et al., 1997). Ocular hemodynamic parameters were measured again during the last 10 min of ET-1 administration. On the second study day the subjects crossed over to the alternative treatment option.

2.4. Measurements

2.4.1. Blood pressure and pulse rate

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

2.4.2. Intraocular pressure (IOP)

Oxybuprocainhydrochloride with fluorescein was instilled for corneal anesthesia. IOP was measured with an applanation-tonometer (Perkins MK2, Clement Clarke, Edinbourgh, United Kingdom).

2.4.3. Blue-field entoptic technique

To quantify retinal leukocyte movements, a commercially available Blue-Field Simulator (Oculix Sarl, Arbaz Switzerland) was used. A detailed description of this noninvasive method was

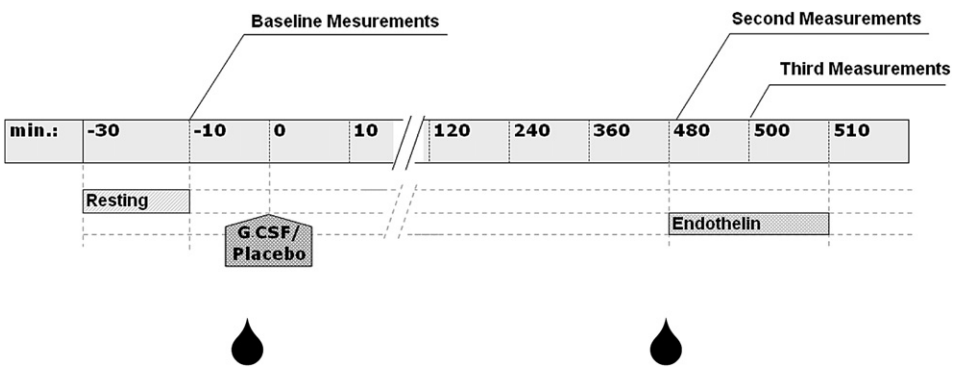

Fig. 1. Time schedule for both study days. Measurements included blue-field entopic technique, laser Doppler flowmetry, laser Doppler velocimetry and dynamic vessel analyzer. Subjects crossed over to second treatment option on second study day (G-CSF or placebo). ♦ Blood was drawn to detect WBC count.