Endothelin-1 Contributes to Hyperoxia-Induced Vasoconstriction in the Human Retina

Susanne Dallinger,1 Guido T. Dorner,1 Roland Wenzel,1 Ursula Graselli,1 Oliver Findl,5 Hans-Georg Eichler,1 Michael Wolzt,1 and Leopold Schmetterer1,2

PURPOSE. There is evidence that ocular blood flow strongly depends on arterial oxygen tension. Results from recent animal studies indicate that the vasoconstrictor response to hyperoxia may be mediated in part by an increased production of endothelin (ET)-1. In an effort to answer the question whether the retinal vasoconstrictive response to hyperoxia in humans is mediated through ET-1, changes in ocular hemodynamics induced by 100% O2 breathing were studied in the absence and presence of an ETα receptor antagonist (BQ-123).

METHODS. The study was a randomized, placebo-controlled, double-masked, balanced, three-way crossover design. On separate study days 15 healthy male subjects received infusions of BQ-123 (either 60 μg/min or 120 μg/min) or placebo. The effects of BQ-123 or placebo on hyperoxia-induced (100% O2 breathing) changes in retinal and pulsatile choroidal blood flow were assessed with the blue-field entoptic technique and with laser interferometric measurement of fundus pulsation, respectively.

RESULTS. During baseline conditions, hyperoxia caused a decrease in retinal blood flow between −29% and −54% (P < 0.001) and a decrease in fundus pulsation amplitude between −7% and −8% (P < 0.001). BQ-123 dose dependently blunted the response to hyperoxia in the retina (60 μg/min: −25%, 120 μg/min: −20%; P = 0.004), but not in the choroid.

CONCLUSIONS. These results indicate that ET-1 contributes to hyperoxia-induced retinal vasoconstriction in the human retina. (Invest Ophthalmol Vis Sci. 2000;41:864–869)

There is evidence from several studies that retinal,1–3 but not choroidal, blood flow4–6 strongly depends on arterial oxygen tension. Hyperoxia induces a pronounced reduction in retinal blood flow, which has been shown in animal7–9 and human experiments.1–3,10 Because both the choroidal and the retinal circulation contribute to the oxygenation of the retina, the oxygen reactivity of these vascular beds seems to be of major importance to the functional integrity of the retina, and alterations in this tightly regulated system may play a pathogenic role in diabetes mellitus. However, the mechanisms underlying hyperoxia-induced vasoconstriction in the retina are not yet clear.

Results from recent animal studies in rats9 and newborn piglets11 indicate that the vasoconstrictor response to hyperoxia may be mediated in part by an increased production of endothelin (ET)-1. In an effort to answer the question of whether the findings can be extrapolated to human physiology, or whether the retinal vasoconstrictive response to hyperoxia is mediated through ET-1 in humans, the ocular hemodynamic response to 100% O2 breathing was studied in the absence and presence of the ETα receptor-selective antagonist BQ-123. The effects of BQ-123 on hyperoxia-induced changes in retinal and pulsatile choroidal blood flow were assessed with the blue-field entoptic technique12 and with laser interferometric measurement of fundus pulsation,13 respectively.

METHODS

Subjects

The study protocol adhered to the tenets of the Declaration of Helsinki. After approval of the study protocol by the Ethics Committee of the Vienna University School of Medicine and after written informed consent was obtained, 15 healthy male subjects were recruited (age: 27.1 ± 3.8 [SD] years). Recruitment was based on the subject’s ability to provide reproducible results with the blue-field entoptic technique. All volunteers passed a prescreening examination during the 4 weeks before the first study day, which included a physical examination and medical history, 12-lead electrocardiogram, complete blood cell count, clinical chemistry, urine drug screen, and an ophthalmic examination. Subjects with normal findings in the screening examinations and ametropia of less than 3 diopters were included in the trial.

The sample size calculation of the present trial was based on an estimated 35% reduction of retinal blood flow in response to hyperoxia (oxygen reactivity). Reproducibility of retinal blood flow measurements with the blue-field entoptic technique in our laboratory was determined previously.14 Using these data, an α error of 5%, and a β error of 10%, we calculated a sample size of 15 healthy subjects.
Study Design

The study was a randomized, placebo-controlled, double masked, balanced, three-way crossover design with a washout period of at least 5 days. On different study days all subjects received infusions of BQ-123 (Clinalfa, Laufenlingen, Switzerland; either 60 μg/min or 120 μg/min) or placebo, in combination with inhaling oxygen (100% O₂; AGA-Gases for Human Use, Vienna, Austria). Subjects were asked to refrain from consuming alcohol and caffeine for at least 12 hours before trial days and were studied after an overnight fast.

Description of Study Days

After subjects had a 20-minute resting period in a sitting position, baseline measurements of fundus pulsation amplitude with laser interferometry and retinal blood flow with the blue-field entoptic technique, blood pressure, and pulse rate were obtained. Thereafter, an inhalation period of 100% oxygen was scheduled for 12 minutes, and hemodynamic measurements were performed during the last 5 minutes. After the inhalation period, a 30-minute resting period was scheduled to restore baseline conditions. At the end of the resting period hemodynamic measurements were performed again. Thereafter, BQ-123 (either 60 μg/min or 120 μg/min) or placebo was administered in a constant intravenous infusion over 32 minutes. Measurements were performed again 15 minutes after the start of the BQ-123 or placebo infusion. After 20 minutes of the infusion, an additional 12-minute inhalation period of 100% oxygen was scheduled, and hemodynamic measurements were performed during the last 5 minutes. Blood pressure was measured in 5-minute intervals throughout the study period. Pulse rate and a real-time electrocardiogram were monitored continuously.

Rationale for BQ-123 as ETₐ Receptor Antagonist and Doses

BQ-123 is a well-characterized ET receptor antagonist with high selectivity for the ETₐ receptor. In a pilot study in five healthy young subjects we investigated the effect of 120 μg/min BQ-123 on thromboxane plasma levels, because thromboxane appears to play an important role in hyperoxia-induced vasoconstriction in the retina of newborn pigs. A 60-minute intravenous infusion of the ETₐ receptor antagonist did not affect thromboxane plasma levels, which were determined with a commercially available radioimmunoassay (baseline: 224 ± 31 pg/ml; 60 minutes: 206 ± 25 pg/ml). Our previous experiments in healthy subjects have shown that 60 μg/min BQ-123 caused no adverse clinical effect but prevented the renal pressor effect of exogenous ET-1. In the present study we anticipated that hyperoxia would induce a marked local increase in ET-1 in retinal vessels because of the pronounced reduction in retinal blood flow observed during 100% O₂ breathing. Thus, we used an even higher dose of BQ-123 to adequately counteract endogenous release of ET-1 during hyperoxia.

Systemic Hemodynamics

Systolic and diastolic blood pressures were measured on the upper arm by an automated oscillometric device. Pulse rate was automatically recorded from a finger pulse-oximetric device (HP-CMS Patient Monitor; Hewlett Packard, Palo Alto, CA). A vice (HP-CMS Patient Monitor; Hewlett Packard, Palo Alto, CA).17

Fundus Pulsation Technique

In all subjects the right eye was studied. Ocular fundus pulsation was assessed by laser interferometry as described by Schmetterer et al. Briefly, the eye is illuminated by the beam of a single-mode laser diode (λ = 783 nm) along the optical axis. The light is reflected at both the front surface of the cornea and the retina. The two re-emitted waves produce interference fringes from which the distance changes between cornea and retina during a cardiac cycle can be calculated. These distance changes are caused by the pulsatile inflow of blood through the arteries and by the nonpulsatile outflow through the veins. The maximum change in corneoretinal distance is called fundus pulsation amplitude (FPA). The method has been shown to estimate the pulsatile blood flow in the choroidal vasculature.

Blue-Field Entoptic Technique

Retinal blood flow was assessed with the blue-field entoptic technique. This noninvasive method is described in detail by Riva and Petrig. The blue-field entoptic phenomenon can be seen best by looking into a blue light with a narrow optical spectrum at a wavelength of approximately 430 nm. Under these conditions tiny corpuscles can be seen flying around swiftly in an area of 10° to 15° of arc radius centered at the fovea. Most likely this phenomenon occurs because red, but not white, blood cells absorb short-wavelength light. Thus, the passage of a white blood cell is perceived as a flying corpuscle. For determination of retinal hemodynamic parameters, a simulated particle field is shown to the subjects under study. By comparison with their own entoptic observation subjects can adjust the number of white blood cells and the mean flow velocity. Retinal blood flow is then calculated as the product of the number of white blood cells and mean flow velocity. The main limitation of this approach is that it has not yet been shown that density of leukocytes is a valid measure of vascular volume under conditions of hyperoxia. We therefore additionally present data on mean flow velocity and leukocyte density separately. Each measurement consisted of at least five matching tests, in which the means of velocity and density were calculated. Only values with an SD of less than 15% were accepted as accurate. Because this depends on the skill of the volunteers, subjects who did not reach an SD lower than 15% at any matching trial during the study were excluded from the trial.

Data Analysis

The effect of BQ-123 was calculated as the percentage of change from baseline values. The mean value of these individual changes was defined as oxygen reactivity and was chosen as the main outcome variable of the present trial. Statistically significant effects of BQ-123 on oxygen reactivity were assessed by comparing the results from the second inhalation periods at all 3 study days with analysis of variance (ANOVA). The effect of BQ-123 on pulsatile choroidal and retinal blood flow alone was analyzed with ANOVA (versus placebo) and expressed as the percentage of change from the pretreatment value. ANOVA was also used to compare baseline oxygen reactivity on the 3 study days and baseline hemodynamic variables. Post hoc analysis was performed with paired t-tests using the Bonferroni correction for multiple comparisons. Data are presented as means ± SEM. P < 0.05 was considered the level of significance.
RESULTS

There were no significant differences among the baseline values on the three trial days (Table 1). BQ-123 and placebo had no consistent effect on systemic hemodynamics (Table 2). In addition, placebo did not affect retinal blood flow, leukocyte velocity, leukocyte density, or FPA.

During baseline conditions hyperoxia caused a significant decrease in FPA, retinal blood flow, and retinal leukocyte velocity, but not retinal leukocyte density on all study days ($P < 0.001$ each). On the 3 study days hyperoxia reduced FPA by $-7\% \pm 1\%$, $-8\% \pm 1\%$, and $-7\% \pm 1\%$. The responses in retinal blood flow to hyperoxia ($-34\% \pm 4\%$, $-29\% \pm 3\%$, and $-30\% \pm 3\%$) and retinal leukocyte density ($-25\% \pm 5\%$, $-22\% \pm 4\%$, and $-23\% \pm 3\%$) were also comparable on the 3 study days.

BQ-123 alone caused an increase in FPA, an indicator of pulsatile choroidal blood flow, of $4\% \pm 1\%$ and of $6\% \pm 2\%$ during the lower and higher doses, respectively ($P = 0.003$ versus placebo, Fig. 1). The lower dose of BQ-123 increased retinal blood flow by $7\% \pm 4\%$ and the higher dose of BQ-123 by $9\% \pm 5\%$. As compared to the increase ($9\% \pm 5\%$) during the placebo period, the effects of BQ-123 on retinal blood flow were not significant ($P = 0.927$ versus placebo, Fig. 1). Accordingly, BQ-123 did not affect leukocyte velocity or leukocyte density.

BQ-123 significantly blunted the response of retinal blood flow and retinal leukocyte velocity to systemic hyperoxia ($P = 0.003$, Fig. 2). The hyperoxia-induced decrease in retinal blood flow was $-25\% \pm 2\%$ during the lower dose of BQ-123 and $-20\% \pm 2\%$ during the higher dose (Fig. 2). As shown in Figure 2 the response in retinal blood flow to 100% O2 breathing was blunted in 11 of 15 subjects during 120 μg/min BQ-123 compared with baseline reactivity. In comparison, no consistently different response pattern to placebo was observed. The hyperoxia-induced decrease in retinal leukocyte velocity was also blunted by BQ-123 ($P = 0.030$). Hyperoxia decreased retinal leukocyte velocity by $-24\% \pm 4\%$ with placebo, by $-16\% \pm 3\%$ with 60 μg/min BQ-123, and by $-13\% \pm 3\%$ with 120 μg/min BQ-123. By contrast, BQ-123 did not alter the FPA response to hyperoxia. The hyperoxia-induced decrease in FPA was $-9\% \pm 1\%$ during the lower BQ-123 dose and $-10\% \pm 1\%$ during the higher BQ-123 dose ($P = 0.586$).

DISCUSSION

The potent vasoconstrictor effect of acute hyperoxia on the retinal vasculature has been shown in numerous animal1–9 and human1–3,10,19,20 studies. The results presented in this report demonstrate a role of ET-1–ET $\alpha$ receptor mediation in the retinal vasoconstrictor response to hyperoxia in humans.

In the present study we observed a decrease in retinal blood flow under systemic hyperoxia between $-29\%$ and $-34\%$ under baseline conditions using the blue-field entoptic technique. This is in good agreement with previous studies investigating retinal blood flow responses to hyperoxia with the same technique.2,21 In other studies,3,10 a reduction of retinal blood flow of $-29\%$ to $-36\%$ was found under hyperoxia by using scanning laser Doppler flowmetry. The response in retinal blood flow to hyperoxia obtained with a combination of laser Doppler velocimetry and fundus camera–based vessel size determination is more pronounced.1,19,20 There is, however, evidence that velocities of erythrocytes and leukocytes in retinal capillaries are different,2,22 and hyperoxia does not necessarily affect erythrocyte and leukocyte movement to the same extent.

By contrast, the response in fundus pulsation amplitude to hyperoxia in the present study was small. Again, this is in

<table>
<thead>
<tr>
<th>Study Day</th>
<th>Placebo</th>
<th>BQ-123 (60 μg/min)</th>
<th>BQ-123 (120 μg/min)</th>
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</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>76.7 ± 2.8</td>
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<td>Pulse rate (beats/min)</td>
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<td>Retinal blood flow (arbitrary units)</td>
<td>110 ± 17</td>
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<td>Velocity (arbitrary units)</td>
<td>107 ± 10</td>
<td>108 ± 9</td>
<td>108 ± 9</td>
</tr>
<tr>
<td>Density (arbitrary units)</td>
<td>3.38 ± 0.26</td>
<td>3.39 ± 0.27</td>
<td>3.39 ± 0.27</td>
</tr>
<tr>
<td>Fundus pulsation amplitude (μm)</td>
<td>0.08 1.07</td>
<td>0.10 0.10</td>
<td>0.10 0.10</td>
</tr>
</tbody>
</table>
| Placebo values are the premedication values 30 minutes after the end of the first oxygen-breathing period. Data are presented as means ± SEM ($n = 15$). Baseline values were compared with ANOVA and the corresponding $P$ is shown.

Baseline values are the premedication values 30 minutes after the end of the first oxygen-breathing period. Data are presented as means ± SEM ($n = 15$). Baseline values were compared with ANOVA and the corresponding $P$ is shown.
accordance with results in a variety of animal and human studies and indicates that oxygen tension has only a minor impact on choroidal blood flow.

In the present study we observed a significant effect of the ETA receptor antagonist BQ-123 on normoxic baseline FPA, but not on retinal blood flow under baseline conditions. Whether this result indicates a different contribution of ET-1 to basal tone in these vascular beds or can rather be attributed to a different reproducibility of the methods used is unclear. Differences in the role of ET-1 on basal blood flow have also been reported between the human forearm and the kidney. ET is known to be crucial in vascular control, and there is increasing evidence that ET-1 also plays a role in ocular blood flow control. ETA receptor subtype binding sites have been identified in retinal arteries and arterioles and in retinal pericytes of animals and humans. The ETA receptor subtype is characterized by its very high affinity for ET-1. Intravitreal injection of ET reduced retinal blood flow in rats and rabbits. Vasoconstrictor effects of ET-1 have also been reported in the optic nerve head and in the choroid in humans. It is conceivable that the ocular vasoconstriction elicited by hyperoxia is partly mediated by ET-1. This concept is supported by the results of the present study, because specific ETA receptor blockade blunted the decrease of retinal blood flow to hyperoxia. The ETA receptor subtype is characterized by its very high affinity for ET-1. Based on our experiments a contribution of ETB receptors to hyperoxia-induced vasoconstriction cannot be excluded. The ETB1 receptor, which is present on endothelial cells, mediates vasorelaxation through the release of nitric oxide. In contrast, the ETB2 receptor mediates direct vasoconstriction and has equal affinity to ET-1 and ET-3. It has recently been shown that ETB receptor blockade induces vasoconstriction in humans. This finding almost precludes a role of ETB receptor subtypes in hyperoxia-induced vasoconstriction in the human retina.

A limitation of the present trial is that we cannot exclude that BQ-123 has some affinity to adrenergic, adenosynergic, or other non-ET receptors. However, there is currently no evidence for unspecific effects of BQ-123 on vascular tone. We have demonstrated recently that intravenous infusion of BQ-123 at a dose of 60 µg/min completely antagonized the vasoconstrictive response of the renal vasculature to exogenous
The results of the present study demonstrate that the hyperoxia-induced reduction of retinal blood flow cannot be fully blocked, even by 120 \( \mu \text{g/min} \) BQ-123. Interestingly, the effect of BQ-123 on hyperoxia-induced vasoconstriction in the retina was dose dependent. Therefore, we cannot exclude the possibility that higher doses of the ETA receptor antagonist would be more effective in blunting retinal oxygen reactivity. Nevertheless, it is unlikely that hyperoxia-mediated retinal vasoconstriction is solely mediated by ET-1 in humans. This is compatible with the results of Zhu et al.\textsuperscript{11} in newborn pigs showing that thromboxane and 20-hydroxyeicosatetraenoic acid also contribute to hyperoxia-induced reductions in retinal blood flow. In addition, there is evidence from in vitro studies on isolated pericytes for an interaction between retinal oxygen tension and the L-arginine/nitric oxide pathway.\textsuperscript{35,36} It remains to be investigated, which mechanisms other than ET-1 release are involved in hyperoxia-induced retinal vasoconstriction in humans.

In conclusion, this is the first human trial to identify one of the mediators of hyperoxia-induced vasoconstriction in the retina. It remains to be established, which factors other than ET-1 play a role in the vasoconstriction induced by high arterial oxygen tension in the human retina.

**References**