Role of Endothelin-1 in Choroidal Blood Flow Regulation during Isometric Exercise in Healthy Humans

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PURPOSE. There is evidence that the choroid has some autoregulatory capacity in response to changes in ocular perfusion pressure (OPP). The mediators of this response are hitherto unidentified. The hypothesis for the current study was that endothelin (ET)-1 and/or angiotensin (ANF)-II may be involved in choroidal vasoconstriction during an increase in OPP.

METHODS. To test this hypothesis a randomized, double-masked, placebo-controlled, three-way crossover study was performed in 12 healthy male volunteers. Subjects received on different study days intravenous infusions of the specific ET_A receptor antagonist BQ-123, the angiotensin converting enzyme inhibitor enalapril or placebo. During these infusion periods subjects were asked to squat for 6 minutes. Choroidal blood flow was measured using a confocal laser Doppler flowmeter and ocular perfusion pressure (OPP) was calculated from mean arterial pressure and intraocular pressure.

RESULTS. BQ-123 and enalapril had no effect on basal blood pressure, pulse rate, intraocular pressure, or choroidal blood flow. During isometric exercise, a pronounced increase in mean arterial pressure paralleled by an increase in OPP was observed. Although choroidal blood flow slightly increased during squatting, the increase was much less pronounced than the increase in OPP, indicating some regulatory potential of the choroid. Enalapril did not alter the choroidal pressure–flow relationship during isometric exercise, but BQ-123 induced a significant leftward shift of the pressure–flow curve ($P < 0.001$).

CONCLUSIONS. The present data indicate that ET-1, but not ANG II, plays a role in choroidal blood flow regulation during isometric exercise in healthy humans. Hence, impaired choroidal autoregulation in patients with ocular vascular diseases may arise from an altered endothelin system. Further studies in such patients are warranted to verify this hypothesis. (Invest Ophthalmol Vis Sci. 2003;44:728–733) DOI:10.1167/iovs.02-0372

There is evidence from various recent animal1–5 and human6–10 studies that the choroid shows some degree of autoregulation. Although autoregulation in the strict sense cannot be directly investigated in humans, evidence that the choroid is not an absolutely passive vascular bed arises from studies using experimental decrease or increase in perfusion pressure. During an experimental increase in perfusion pressure, net choroidal vasoconstriction is required to keep blood flow constant. Numerous mediators may be involved in this vasoconstrictor response, because blood flow in the choroid is controlled by numerous hormonal, neural, and paracrine regulatory systems.11 In the present study we tested the hypothesis that endothelin (ET)-1 and/or angiotensin (ANG)-II may be involved in choroidal vasoconstriction during isometric exercise.

ET-1 is the most potent vasoconstrictor known and is produced predominantly by the vascular endothelium. The peptide binds to the ET_A receptor located on vascular smooth muscle cells that mediate vasoconstriction and to the ET_B receptor located on endothelial cells that mediate vasodilation through the release of nitric oxide (NO) and prostacyclin.12 In the human choroid, the potent vasoconstrictor response to ET-1 is mainly mediated by the ET_A receptor.13 In vitro,14,15 animal16–20 and human11–12,21 studies indicate that ET-1 is a major determinant of choroidal blood flow.

Whether the potent vasoconstrictor ANG II is a major determinant of choroidal blood flow is not established. ANG II induces contraction in isolated human posterior ciliary arteries.24 However, the ANG II receptor antagonist losartan did not alter the choroidal pressure–flow relationship in the rabbit.16 In addition, pharmacologic stimulation or inhibition of angiotensin receptors had no effect on choroidal blood flow in healthy humans.25,26

To test the study hypothesis we performed a study in which choroidal flow–pressure relationships were compared in the absence or presence of the ET_A receptor antagonist BQ-123 and the angiotensin-converting enzyme (ACE) inhibitor enalapril. Perfusion pressure was calculated from measurements of arterial blood pressure and intraocular pressure (IOP). Choroidal blood flow was measured using a recently developed portable laser Doppler flowmeter.27,28

METHODS

Subjects

The present study was performed in compliance with the Declaration of Helsinki and the Good Clinical Practice guidelines. After approval of the study protocol by the Ethics Committee of the Vienna University School of Medicine and after written informed consent was obtained, 12 healthy nonsmoking male subjects were studied (age: 19–31 years, mean 23 ± 4 years [SD]). All subjects were drug-free for at least 3 weeks before inclusion and passed a prestudy screening during the 4 weeks before the first study day that included medical history and physical examination, 12-lead electrocardiogram; complete blood count; activated partial thromboplastin time; thrombin time; clinical chemistry (sodium, potassium, creatinine, uric acid, glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate transcarbamylase, γ-glutamyltransferase, alkaline phosphatase, total bilirubin, and
total protein), hepatitis A, B, and C, and HIV-serology; urinalysis; and
an ophthalmic examination. Subjects were excluded if any abnormality was
found as part of the pretreatment screening, unless the investiga-
tors considered an abnormality to be clinically irrelevant. In addition, subjects
with asthenopia of more than 3 D, anisometropia of more than 1 D, or any
evidence of eye disease that might interfere with the purpose of the present trial were excluded. During the week after completion of the study, a follow-up safety investigation was scheduled for
all subjects. This follow-up investigation included complete blood
count, activated partial thromboplastin time, thrombin time, clinical
chemistry (sodium, potassium, creatinine, uric acid, glucose, choles-
terol, triglycerides, alanine aminotransferase, aspartate transcarbamy-
lase, γ-glutamyltransferase, alkaline phosphatase, total bilirubin, and
total protein), and urinalysis.

Study Design

Subjects were asked to refrain from alcohol and caffeine for at least 12
hours before trial days and were studied after an overnight fast. The
study followed a randomized double-masked placebo-controlled three-
way crossover design with BQ-123 (Cilinalfa, Läufelfingen, Switzerland;
dose: 60 μg/min over 60 minutes), enalapril (Renitec; MSD, Haarlem,
the Netherlands; dose: 4 mg over 60 minutes), or placebo (physiologic
saline solution). All drugs were administered intravenously into an
antecubital vein, with automated devices used to ensure constant
infusion rates.

Rationale for Drug and Doses

BQ-123 is a well characterized cyclic pentapeptide endothelin antago-
nist with high ET₂ selectivity.²⁹ BQ-123 has been shown to induce
dose-dependent vasodilation in the human forearm.³⁰ We have per-
formed several studies of intravenous BQ-123 in healthy humans.
Whereas BQ-123 at a dose of 60 μg/min did not affect basal choroidal
and renal blood flow, it reversed the effect of exogenous ET-1 and the
nitric oxide synthase inhibitor Nω-monomethyl-arginine (L-NAME) in
these vascular beds.¹³,³¹,³² In addition, BQ-123 was able to blunt the retinal hemodynamic response to hyperoxia in the human retina.³³
Enalapril is a well characterized ACE inhibitor. From a pharmaco-
logic point of view, it would have been preferable to use an ANG II
receptor antagonist such as losartan. However, currently no ANG II
receptor antagonist is available for intravenous use in humans. We
therefore decided to use the ACE inhibitor enalapril to allow for
double-masked conditions, although this drug also acts through the
bradykinin pathway. The dose of enalapril was selected based on an
to early trial in healthy humans.³⁴

Description of Study Days

Three different study days were scheduled for each subject. After a
resting period of at least 20 minutes, which was scheduled to ensure
costant hemodynamic conditions, baseline measurements of ocular
and systemic hemodynamics were performed. Choroidal blood flow
was measured continuously for 3 minutes at baseline, using laser
doppler flowmetry (LDF). Thereafter, subjects were asked to squat for
6 minutes and choroidal blood flow was measured continuously with
LDF. Squatting was performed in a position in which the upper and the
lower leg were as close as possible to a right angle. For the subject’s
security a nurse stood behind each subject during the squatting peri-
dods. Systemic hemodynamics were assessed every minute during the
squatting period. The IOP was measured at baseline and at the end of the
squatting period. Thereafter, a resting period of at least 50 minutes
was scheduled. When systemic hemodynamics had returned to base-
line, administration of the drug was started. Fifty-one minutes after the
start of infusion measurement of choroidal blood flow was started and
continuously continued for 3 minutes at baseline. Fifty-four minutes
after the start of drug administration another squatting period was
scheduled for all subjects. This second period of isometric exercise
was identical with the one before the drug was administered. Subjects
crossed over to the alternative drugs under study with a washout
period.

Experimental Procedure

Systemic Hemodynamics. Systolic (SBP) and diastolic (DBP)
pressure and mean arterial pressure (MAP) were measured on
the upper arm by an automated oscillometric device. Pulse rate (PR)
was automatically recorded from a finger pulse oximeter (HP-CMS
patient monitor; Hewlett Packard, Palo Alto, CA).

Applanation Tonometry and Ocular Perfusion Pressure.
The IOP was measured with a Perkins applanation tonometer
(Clement Clarke, Edinburgh, UK). Oxybuprocaine hydrochloride was
used to anesthetize the cornea. Ocular perfusion pressure was calcu-
lated as OPP = MAP \ − \ IOP.§ When isometric exercise we
observed only small changes of IOP over baseline after 6 minutes of
squatting. Hence, we used a linear regression model to extrapolate the
IOPs at the other time points of squatting.

Laser Doppler Flowmetry. Continuous measurement of cho-
roidal blood flow (CBF) was performed with LDF.³⁶ With this tech-
nique, the vascularized tissue is illuminated by coherent laser light.
Light scattered by the moving red blood cells undergoes a frequency
shift. In contrast, static tissue light-scattering cells do not change the
light frequency, but lead to randomization of light directions, imping-
ing on red blood cells. Hence, red blood cells receive light from
numerous random directions. Because the frequency shift is depend-
ent not only on the velocity of the moving red blood cells, but also on
the angle between the wave vectors of the incident and the scattered
light, scattering of the light in tissue broadens the Doppler shift power
spectrum. From this spectrum the average velocity of the red blood
cells and blood volume can be determined based on a theory of
light-scattering in tissue.³⁷ CBF is then calculated as the product of
average velocity of the red blood cells and blood volume.

In the present study, a compact laser Doppler flowmeter, which has been described in detail,²⁷,²⁸ was used for the measurements of the
CBF. The laser beam of a single-mode laser diode (785 nm, 90 μW at
the cornea) is delivered to the eye through a confocal optical system.
The beam diameter at the fundus is nominally 12 μm. The light is
collected by a bundle of six fibers with a core diameter of 110 μm,
which are arranged on a circle with a diameter of 180 μm. All mea-
surements were performed in the fovea by asking the subject to
directly fixate the beam, which appeared as a small red dot. The fovea
was chosen, because the retina is avascular in this region.

Compared with previous fundus camera-based systems,²⁶ for the
assessment of choroidal blood flow, the new system offers two major
advantages. Adjustment of the detector in relation to the measurement
on the retina is omitted, because the system uses confocal optics. In
addition, the system is portable, which facilitates measurements during
isometric exercises.

Data Analysis

All statistical analyses were performed on computer (Statistica, ver.
4.5; StatSoft, Inc., Tulsa, OK). All outcome variables were calculated for
each subject individually and then averaged. The effect of exercise on
the outcome parameters was assessed with repeated measures ANOVA. The effect of BQ-123 and enalapril on baseline parameters was
compared using two-way ANOVA. The relative change in he-
monodynamic parameters induced by isometric exercise was calculated.
For the experiments during BQ-123, enalapril, or placebo the value imme-
diately before the start of isometric exercise was taken as baseline. To
gain information on the pressure-flow relationship relative data were
sorted according to ascending OPP.²⁷ For each squatting period, we
obtained a total of 72 OPP and CBF readings. These were divided into
eight groups of nine OPPs and CBFs. Hence, the first group consisted
of the data with the lowest relative OPP values (n = 9) and the eighth
group of the data with the highest relative OPPs (n = 9). The mean
values from these groups were used to determine the OPP at which the
CBF significantly deviated from baseline. This was the case when the
RESULTS

All drugs were well tolerated, and no adverse events were reported. Baseline data on the 3 days of the trial were comparable. Isometric exercise significantly increased MAP and PR during all baseline squatting periods \((P < 0.001, \text{Figs. 1 and 2})\), and the systemic effect was comparable on all study days. Isometric exercise caused only small changes in IOP (Table 1), which did not reach the level of significance. Accordingly, OPP was mainly influenced by changes in MAP and increased significantly during all squatting periods (Fig. 3). During all squatting periods, we observed an increase in CBF which was, however, much smaller than the increase in OPP. The increase in CBF, which was at its maximum between 12% and 20%, was significant versus baseline \((P = 0.005, \text{Figs. 4 and 5})\), but not different between trial days.

Placebo, BQ-123, and enalapril had no consistent effect on blood pressure, PR, or IOP (Figs. 1, 2, Table 1) at baseline. Similarly, none of the administered drugs affected basal CBF (Fig. 4). The response of OPP and CBF to isometric exercise was not altered by placebo infusion (Figs. 4, 5). BQ-123 and enalapril did not affect the squatting-induced increase in OPP, as shown in Figure 1. By contrast, BQ-123 altered the time course of CBF \((P = 0.0014)\) during isometric exercise. Enalapril did not alter the time course of CBF during squatting (maximum increase in CBF: 14%). The maximum increase in CBF during isometric exercise was 28% when BQ-123 was infused, but only 15% when placebo was administered.

The pressure-flow relationships as calculated from the categorized data are presented in Figure 6. No differences were observed in the pressure-flow data under basal conditions. Before administration of the drug, CBF increased at OPPs between 61% and 67% above baseline. When placebo or enalapril were administered, the pressure-flow relationship in the choroid was not significantly altered. CBF started to increase over baseline values at OPPs of 65% and 67%, respectively. By contrast, the choroidal pressure-flow relationship was significantly altered when BQ-123 was administered. During ET\(_A\) receptor blockade, a statistically significant leftward shift of the pressure flow relationship was observed. When isometric exercise was performed during administration of BQ-123, CBF had already started to increase at an OPP of 50% above baseline \((P < 0.001 \text{ vs. placebo})\).

DISCUSSION

The findings in the present study suggest that ET-1, but not ANG II, plays a role in regulation of CBF during isometric exercise. Accordingly, ET-1 may be released during squatting, presumably from the vascular endothelium, and induces vaso-
constriction to counteract the increase in perfusion pressure. Because the ACE inhibitor enalapril did not alter the time course of CBF during isometric exercise and the pressure-flow relationship, ANG II does not appear to contribute to the increase in vascular resistance in the face of the changes in perfusion pressure.

In the rabbit model of Kiel\textsuperscript{18,19} the specific ET\textsubscript{a} receptor antagonist FR-139317 did not alter the pressure-flow relationship when the OPP was mechanically altered. By contrast, specific ET\textsubscript{b} receptor blockade using A-192621 induced a small leftward shift in the choroidal pressure-flow relationship. Kiel speculated that this may be due to different receptor sensitivities in the choroid. The differences between the present study and the rabbit experiments may be related to several factors. On the one hand, species-specific regulation in choroidal blood flow regulation may play a role. On the other hand, isometric exercise is not directly comparable to a mechanical increase in OPP in the rabbit, because a number of non-pressure-related effects, including modulation of neural input of the choroid, may occur.

The present study did not address the potential role of the ET\textsubscript{a} receptor in regulation of choroidal blood flow. As mentioned earlier, there is some evidence from animal studies for involvement of the ET\textsubscript{a} receptor in regulation of ocular blood flow. We are not aware however, of any human studies that have investigated the role of this receptor subtype in the control of ocular blood flow. Obviously, additional studies are needed to elucidate this question in the near future.

The present study supports the concept that the human choroid is autoregulated, but it does not necessarily verify it, because squatting induces sympathetic and parasympathetic stimulation\textsuperscript{38} in addition to the increase in OPP. Nevertheless, our data clearly indicate an increase in choroidal vascular resistance during isometric exercise, because the change in CBF was much less pronounced than the change in OPP. Compared with previously published studies that assessed choroidal pressure-flow relationships during isometric exercise\textsuperscript{7,9} the present results during squatting indicate a slightly higher regulatory capacity of the choroid. The reason for this discrepancy may be related to interindividual differences in the MAP increase during squatting, but also to interindividual differences in the autoregulatory capacity of the choroid.

The observation that ET-1 may be involved in regulation of choroidal blood flow during changes in OPP may be of clinical relevance, because ET-1 has been implicated in a variety of ocular diseases with vascular involvement. Our results are well compatible with the results of Hasler et al.\textsuperscript{10} who have shown that choroidal autoregulation is altered in patients with acral vasospasm. Considering that ET-1 is assumed to play a key role in vasospastic episodes, future studies are warranted to elucidate a possible link between an altered ET-1 system and impaired choroidal autoregulation in patients with vasospasm. ET-1 has also been implicated in the pathogenesis of glaucoma\textsuperscript{39} and may provide a link between altered blood flow regulation and increased IOP. The results of the present trial are compatible with an altered ET system in glaucoma, in that

**FIGURE 4.** Effect of squatting on CBF. Symbols and data presentation are as in Figure 1. #Significant changes versus baseline; *significant effects of BQ-123 on exercise-induced changes in CBF versus placebo.

**FIGURE 5.** Relative change of CBF over the pre-exercise level during squatting. Symbols and data presentation are as in Figure 1. #Significant changes versus baseline; *significant effects of BQ-123 on exercise-induced changes in CBF versus placebo.

**FIGURE 6.** Pressure-flow relationship, determined by the categorized ocular perfusion pressure (OPP) and choroidal blood flow (CBF) during isometric exercise. Relative data were sorted into groups of nine values, each according to ascending OPP. The first period of squatting was without the drug (baseline; open triangles). The second squatting period was during administration of placebo, BQ-123, or enalapril (filled triangles). The mean and the lower limits of the 95% confidence interval are shown (n = 12).
several investigators have reported signs of altered autoregulation in glaucomatous subjects. Future studies are needed to investigate whether modulation of the ET pathway may be sufficient to normalize ocular blood flow and its regulation in patients with glaucoma. ET-1 has also been implicated, however, in the pathogenesis of other eye diseases, such as diabetic retinopathy and HIV-related retinopathy. However, regulation of choroidal blood flow during changes in OPP has not been thoroughly studied in these diseases.

When interpreting the results of the present study, the limitations of LDF for the measurement of choroidal blood flow have to be considered. On the one hand, only submacular choroidal blood flow is assessed with this technique, and there may well be regional differences in regulation of choroidal blood flow. On the other hand, it is not entirely clear whether the theory of light-scattering in tissue underlying the calculation of choroidal blood flow is applicable to the human choroid, because of the specific angiarchitectures of this vascular bed. Validation of the technique is difficult because no gold-standard method is available for the measurement of choroidal blood flow in humans. There are, however, several lines of evidence showing that LDF data as obtained in the human choroid are closely related to blood flow in the submacular area. There is little reason to breathe 100% oxygen, compatible with the idea that choroidal blood flow is insensitive to changes in arterial oxygen tension. In addition, there is a dose-dependent increase in the choroidal LDF signal, with increasing arterial carbon dioxide tension, which is an important determinant of choroidal blood flow. Riva et al. have shown that the choroidal LDF signal goes to zero when blood flow is stopped by a short-term increase in IOP, showing that the system has no zero offset.

In conclusion, the present study confirms that the vascular resistance is altered in the human choroid during changes in OPP. When the OPP is increased by means of isometric exercise, the choroid reacts with vasoconstriction. This vasoconstrictor response seems to involve ET-1 but not ANG II in the normal human choroid.

References