Effect of Nifedipine on Choroidal Blood Flow Regulation during Isometric Exercise

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PURPOSE. To determine whether nifedipine, an L-type calcium channel blocker, alters choroidal blood flow (ChBF) regulation during isometric exercise in healthy subjects.

METHODS. The study was carried out in a randomized, placebo-controlled, double-masked, two-way crossover design. Fifteen healthy male subjects were randomly assigned to receive either placebo or nifedipine on two different study days. Subfoveal ChBF was measured with laser Doppler flowmetry while the study participants performed isometric exercise (squatting). This was performed before drug administration and during infusion of nifedipine and placebo, respectively. Mean arterial pressure (MAP) and intraocular pressure (IOP) were measured noninvasively, and ocular perfusion pressure (OPP) was calculated as 2/3 MAP − IOP.

RESULTS. MAP and OPP increased significantly during all squatting periods (P < 0.01). The increase in ChBF was less pronounced than the increase in OPP during isometric exercise. Nifedipine did not alter the OPP increase in response to isometric exercise, but it significantly augmented the exercise-induced increase in ChBF (P < 0.001 vs. placebo). Although ChBF increased by a maximum of 14.2% ± 9.2% during the squatting period when placebo was administered, the maximum increase during administration of nifedipine was 23.2% ± 7.2%.

CONCLUSIONS. In conclusion, the data of the present study suggest that nifedipine augments the ChBF response to an experimental increase in OPP. In addition, it confirms that the choroidal vasculature has a significant regulatory capacity over wide ranges of OPPs during isometric exercise. (ClinicalTrials.gov number, NCT00280462.) (Invest Ophthalmol Vis Sci. 2012;53:374–378) DOI:10.1167/iovs.11-8536

A utoregulation is usually defined as the ability of a vascular bed to keep its blood flow constant despite changes in perfusion pressure. There is general agreement now that both retinal and optic nerve head (ONH) blood flow are well auto-regulated.1,2 The situation for the choroid is less clear, though data from several human and animal studies have shown that choroidal blood flow (ChBF) shows at least some degree of blood flow regulation in response to changes in perfusion pressure.3–5

Although some mediators involved in the regulatory processes of choroidal blood flow during isometric exercise have been identified, the exact mechanism underlying the ability of the choroid to change its vascular tone in response to changes in perfusion pressure is still unclear. As such, it has been shown that inhibition of nitric oxide synthase (NOS) alters the response of ChBF to isometric exercise, indicating that NO is part of this process.6 Additionally, evidence has been provided that endothelin-1, a potent endothelium-derived vasconstrictor, is involved in choroidal blood flow regulation during isometric exercise.7,8

Because of their important function in regulating smooth muscle function, calcium channels may well play a role in the regulation of choroidal blood flow during isometric exercise as well. Indeed calcium channel blockers lead to direct vasodilation and trigger the release of NO.9 A number of experiments in the animal eye indicate that calcium channel blockers may increase blood flow,10–12 but little information is available about whether they modify the pressure/flow relationship during changes in perfusion pressure. Verapamil, however, reversed the downward shift induced by NOS inhibition in the choroidal pressure/flow relationship in the rabbit.13

The present study aimed to investigate whether nifedipine, an L-type calcium channel blocker, modifies ChBF regulation during isometric exercise. To test this hypothesis, the ChBF response to isometric exercise was measured in the presence or absence of nifedipine in a group of young, healthy subjects.

SUBJECTS, MATERIALS, AND METHODS

Subjects

The present study was conducted in compliance with the Declaration of Helsinki and the Good Clinical Practice guidelines of the European Union. The study protocol was approved by the Ethics Committee of the Medical University of Vienna. Fifteen healthy male subjects participated in the present study (age, 25.7 ± 3.8 years; mean ± SD) after written informed consent was given.

The sample size calculation was based on data from previous studies performed in our laboratory.6,8,14 which investigated the pressure-flow relationship during isometric exercise using laser Doppler flowmetry (LDF).15 These data were used for the sample size calculation selecting an α-level of 0.05 and a β-level of 0.2. A change in the upper limit of choroidal autoregulation of 10% in ocular perfusion pressure was assumed to be relevant.

During the 4 weeks before the first study day, prestudy screening was carried out. This included physical examination and medical history, vital signs, 12-lead electrocardiography, determination of height and weight, hematologic status (hemoglobin, hematocrit, red blood cell count, and platelet count).

Clinical Trials

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cells [RBC], mean cell hemoglobin, white blood cells [WBC], platelet count, activated partial thromboplastin time, thrombin time), clinical chemistry (fasting blood glucose, sodium, potassium, creatinine, glutamine pyruvate transaminase [alanine aminotransferase], γ-glutamyl transpeptidase, total bilirubin, total protein), urinalysis (WBC, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood/hemoglobin), and an ophthalmic examination.

If any abnormality was found as part of the pretest screening, the subject was not included unless the investigators considered an abnormality to be clinically irrelevant. In addition, only subjects with ametropia of <±5 diopters were allowed to participate in the present study.

Study Design
The study design was randomized, double-masked, placebo-controlled, two-way crossover. Subjects were assigned to receive intravenous infusions of either nifedipine or physiologic saline solution as placebo on two different study days. A minimum washout-period of 4 days was scheduled. Before the trial days, subjects had to abstain from caffeine- or alcohol-containing beverages for at least 12 hours. After 20 minutes of rest, baseline measurements of ocular and systemic hemodynamics were performed. ChBF was measured continuously for 3 minutes at baseline in the sitting position. Thereafter, the chair was removed so that the subjects performed squatting for 6 minutes, and ChBF was measured continuously. Squatting was performed in a position in which the upper and the lower leg formed a right angle as closely as possible. Systemic hemodynamic parameters were assessed every minute. IOP was measured at the end of the squatting period. Thereafter a resting period of at least 30 minutes was scheduled until systemic hemodynamics had returned to baseline. Then drug administration was started. During the last 6 minutes of drug administration, another squatting period was performed. Again, before the squatting period, 3 minutes of baseline measurement were carried out.

Drugs and Drug Administration
Nifedipine (Adalat; Bayer, Leverkusen, Deutschland) was administered with an infusion period of 30 minutes (intravenously). The dose was a 15-μg/kg bolus infusion over 5 minutes and a 0.2-μg/kg(min) maintenance dose with an infusion period of 25 minutes. Physiologic saline solution (as placebo) was administered with an infusion period of 30 minutes. To allow for double-masked conditions, placebo was prepared in two syringes and was subsequently infused.

Methods
Noninvasive Measurement of Systemic Hemodynamics. An automated oscillometric device was used to measure systolic, diastolic, and mean arterial pressure (SBP, DBP, MAP) on the upper arm. Concomitantly, pulse rate (PR) was recorded from a finger pulse-oxymetric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA).

Laser Doppler Flowmetry. Compact LDF was used to measure ChBF. The device can be mounted on a commercially available slit lamp. With this technique, the vascularized tissue is illuminated by coherent laser light. The scattering on moving RBCs leads to a frequency shift in the scattered light. Contrarily, static components in tissue do not change light frequency but lead to randomization of light directions impinging on RBCs. Mean RBC velocity (VEL), blood volume (VOL), and blood flow (FLOW) can be calculated in relative units from the Doppler shift power spectrum. LDF was performed in the fovea to assess ChBF. This was achieved by asking the subject to fixate the laser beam, which appears as a weak red spot. Fixation losses or blinks can easily be identified in the signal; the respective parts of the FLOW readings were not used for analysis.

IOP and OPP. To measure IOP, a slit lamp–mounted Goldmann applanation tonometer was used. To achieve local anesthesia of the cornea, 1 drop of 0.4% benoxinate hydrochloride combined with 0.25% sodium fluorescein was used before each measurement. OPP was estimated as ⅔ MAP – IOP.

Statistical Analysis
The effect of nifedipine on the ChBF blood flow response to isometric exercise was compared with baseline and with placebo. This was done with ANOVA for repeated measurements and planned comparisons as post hoc testing. In addition, the effect of nifedipine on basal ChBF was assessed. To gain information on the pressure-flow relationship, relative data were sorted according to ascending OPP values. For each squatting period, we obtained a total of 90 OPP and CBF data. These were divided into 10 groups of 9 OPP and CBF values. Hence, the first group consisted of the data with the lowest relative OPP values (n = 9), and the tenth group consisted of the data with the highest relative OPP values (n = 9). 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 95% confidence interval did no longer intersect with the baseline level. Data are presented as mean ± SEM. All statistical analyses were carried out using data mining and statistical analysis software (CSS Statistica, version 6.0; StatSoft, Tulsa, OK).

RESULTS
All medications were well tolerated by the subjects. No significant differences in baseline values during the pretreatment periods between the 2 study days were observed (Tables 1 and 2). MAP significantly increased in response to isometric exercise during the pretreatment periods (P < 0.001 vs. baseline), whereas no effect on IOP was observed. This effect was comparable on both study days (P = 0.61 between study days). Accordingly, OPP increased significantly during isometric exercise (P < 0.001 vs. baseline; Fig. 1). The maximum increase in OPP was between 58.9% ± 6.5% and 57.2% ± 5.8% (P = 0.85 between study days). This increase in OPP during the pretreatment squatting periods was accompanied by a slight but significant increase in ChBF (P = 0.75 between study days; Fig. 2). The increase in ChBF (maximum increase, 10.3% ±

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6.2% and 18.3% \pm 8.5%) was, however, less pronounced than the increase in OPP (P = 0.04 vs. baseline). Administration of nifedipine did not alter IOP, SBP, DBP, HR, MAP, OPP, or ChBF at baseline (Tables 1 and 2). Administration of nifedipine tended to decrease MAP, DBP, and OPP, but this effect did not reach the level of significance. The response of OPP and ChBF to isometric exercise was not altered by the administration of placebo (Figs. 1, 2). Although the administration of nifedipine did not change the response of OPP during isometric exercise, it augmented the exercise-induced increase in ChBF (Fig. 2; P < 0.001 vs. placebo; ANOVA). Although ChBF increased by a maximum of 14.2% \pm 9.2% during the infusion of placebo during the squatting period, the maximum increase during the administration of nifedipine was 23.2% \pm 7.2%. Post hoc analysis revealed that this effect was significant during the last 3 minutes of squatting (Fig. 2).

**DISCUSSION**

The results of the present study confirm findings from previous experiments that the choroid regulates its blood flow over a
wide range of OPPs during isometric exercise.\(^2,5,21\) In addition, our data indicate that nifedipine, a calcium channel blocker, alters the response of ChBF during an experimental increase in OPP.

Previous studies indicate that calcium channel blockers can alter ocular blood flow. Nicardipine increased ONH but not retinal blood flow in cats.\(^10\) An increase in ONH blood flow was also found after the administration of lomerizine and nilvadipine in rabbits.\(^11\) Additionally, nilvadipine increased blood velocity in the ONH, choroid, and retina of rabbits.\(^12\) In healthy subjects, oral administration of nimodipine increased retinal blood flow significantly.\(^22\)

The data of the present study show that nifedipine significantly increased ChBF in response to squatting. Interestingly, however, the drug did not increase blood flow at baseline, possibly because of the relatively low dose used. Our data therefore indicate that a vasoconstrictor is endogenously produced during squatting. Obviously, this vasoconstrictive action is counteracted by the calcium channel blocker.

There is evidence that endothelin-1 is involved in mediating this choroidal vasconstrictor response.\(^8,23\) In an earlier study, endothelin-A receptor blockade shifted the choroidal pressure-flow curve to the left.\(^3\) In the present study, the previously reported endothelin-1 antagonizing effect of calcium-channel antagonists might have caused the same alteration in the pressure-flow curve.\(^24,25\) This effect could have been enhanced by a nifedipine-induced release of NO.\(^7,26\) This hypothesis is supported by a study\(^6\) in which the blockade of NOS shifted the choroidal pressure-flow curve to the right. Obviously, a study in humans cannot finally answer the source of endothelin release during isometric exercise. Interestingly, it has been reported that plasma endothelin-1 increases during isometric exercise.\(^26\) Whether this is related to alterations in shear stress associated with the increase in blood pressure\(^27\) or reflects the interaction between the endothelin system and sympathetic stimulation\(^28\) is unclear. Alternatively we cannot exclude that the release of endothelin during isometric exercise comes from ocular tissues. The precise source of endothelin in the posterior segment remains unclear, but the retinal pigment epithelium secretes endothelin-1 toward the basolateral side and therefore may play a role in the regulation of choroidal blood flow.\(^29\) Independently of the source of endothelin, it has been shown that the peptide activates L-type channels in vascular smooth muscle cells.\(^30\) As such, the present data are well compatible with the idea that endothelin plays a part in the choroidal vasoconstrictor response during an isometric exercise-induced increase in OPP.

There are some limitations of the present study in humans that must be mentioned. Subjects started at different baseline OPPs, and wide variability in exercise-induced changes in MAP was observed. We used a crossover design to minimize this problem so that every subject served as his own control. Another limitation was that LDL only measures ChBF in the subfoveal choroid, making the findings of the present study not applicable to peripheral areas of the choroid.\(^31\)

In conclusion, the data of the present study suggest that nifedipine augments the ChBF response to an experimental increase in OPP. Further, it confirms that the choroidal vasculature has a significant regulatory capacity over wide ranges of OPPs during isometric exercise.

**References**


