Effect of Unspecific Inhibition of Cyclooxygenase by Indomethacin on Retinal and Choroidal Blood Flow

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PURPOSE. Animal studies suggest that retinal and choroidal blood flow decrease after administration of indomethacin, a nonspecific cyclooxygenase inhibitor. Cyclooxygenase is the key enzyme involved in the arachidonic pathway and regulates the production of vasoactive substances such as prostaglandins and thromboxans. The aim of the present study was to investigate the short-term effects of indomethacin on ocular blood flow in healthy humans.

METHODS. A randomized, double-masked, placebo-controlled, two-way crossover study in 12 healthy, male, nonsmoking subjects was performed. Indomethacin was administered as a bolus injection of 0.4 mg/kg followed by continuous infusion of 0.4 mg/kg/h over 120 minutes. Ocular hemodynamic parameters were measured at baseline and up to 3 hours after start of the infusion. Subfoveal choroidal blood flow and fundus pulsation amplitude were measured with laser Doppler flowmetry and laser interferometry, respectively. Retinal vessel diameters were assessed using a retinal vessel analyzer. Retinal blood flow was calculated based on retinal vessel diameters, and red blood cell velocity was measured with laser Doppler velocimetry.

RESULTS. Administration of indomethacin decreased retinal arterial diameters up to −4.3% ± 3.4% and reduced retinal blood velocity by a maximum of −29% ± 20% (P < 0.05). Calculated retinal blood flow decreased by −27% ± 21% (P < 0.05), reaching the maximal decrease 60 minutes after administration. Choroidal blood flow and fundus pulsation amplitude (FPA) also decreased during the infusion of indomethacin with maximum effects of −17% ± 13% (P < 0.05, vs. placebo) and −7% ± 4% (P < 0.05, vs. placebo), respectively.

CONCLUSIONS. Results showed a marked decrease in retinal and choroidal blood flow after short-term administration of indomethacin. Whether this decrease can be attributed to a reduced production of prostaglandins or an unknown mechanism has yet to be clarified. Further studies appear to be indicated to investigate whether the long-term intake of indomethacin is associated with an increased risk for vascular eye disease. (Invest Ophthalmol Vis Sci. 2008;49:1065–1070) DOI: 10.1167/iovs.07-0824

Cyclooxygenase (COX) is the key enzyme for the production of several potent vasoactive substances, including the prostaglandins (PGs) and thromboxans. These substances are derived from oxygenation and cyclization of polyunsaturated acids (predominantly arachidonic acid) through well-identified bioenzymatic pathways. PGs have been shown to participate in the regulation of several physiological and pathologic reactions, such as inflammation, edema, and platelet aggregation.

Furthermore, PGs play an important role in the regulation of vascular tone and represent some of the most potent mediators in local blood flow regulation.1–3 Based on the finding that prostacyclin (PGI2, epoprostenol) is endogenously produced by the vascular endothelium of ocular vessels,5 it has been hypothesized that PGs may also contribute to ocular blood flow regulation. Many animal studies showed an influence of prostaglandins on regional ocular blood flow4,6 and on the tone of isolated ophthalmic, ciliary, and retinal arterioles.7,8

Indomethacin is a nonselective COX inhibitor and one of the most commonly used drugs for the treatment of chronic inflammation and pain resulting from rheumatic disease. A reduction of brain, renal, and mesenteric blood flow has been described after administration of the COX inhibitor indomethacin.9–11 Data obtained from animal experiments suggest that retinal and choroidal blood flow react in a similar way in response to indomethacin: A reduction of ocular blood flow after administration of the COX inhibitor indomethacin was observed in several studies.5,6,12

Most of the available data are derived from animal or in vitro studies, and little information is available on the effect of prostaglandins on ocular blood flow in humans. In the present study, we hypothesized that intravenously administered indomethacin may also reduce ocular blood flow in healthy humans.

MATERIALS AND METHODS

Subjects

Twelve healthy, male, nonsmoking volunteers were included (age range, 20–35 years; mean, 26 years; SD, 5 years). The nature of the study was explained, and all subjects signed a written informed consent to participate. The study protocol was approved by the Ethics Committee of the Medical University of Vienna and followed the guidelines of Good Clinical Practice and the Declaration of Helsinki. Each subject passed a screening examination, including medical history and physical examination, 12-lead electrocardiogram, complete blood count, activated partial thromboplastin time, thrombin time, fibrinogen, clinical chemistry (sodium, potassium, creatinine, uric acid, glucose, cholesterol, triglycerides, alanine amino transferase, aspartate transcarbamylase, glutamyl transferase, alkaline phosphatase, total bilirubin, total protein, hepatitis A, B, and C, HIV-serology, urinalysis, and a urine drug screening. Subjects were excluded if any abnormality was found as part of the pretreatment screening, unless the investigators

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considered the abnormality to be clinically irrelevant. Moreover, an ophthalmic examination, including slit lamp biomicroscopy and indirect funduscopy, was performed. Inclusion criteria were normal ophthalmic findings, ametropia of less than 3 diopters (D), and anisometropia of less than 1 D.

**Study Design**

Subjects were studied in a randomized, balanced, double-masked, two-way, crossover design. Two study days were scheduled for each subject, with washout periods of at least 7 days between study days. All subjects were studied with dilated pupils after instillation with tropicamide (Hydriatricum Agepha; Agepha, Vienna, Austria). A time schedule is given in Figure 1. After a 20-minute resting period in a sitting position, baseline measurements were taken of arterial blood pressure and pulse rate. Then subfoveal choroidal blood flow and fundus pulsation amplitude were measured with laser Doppler flowmetry and laser interferometry, respectively. Retinal arterial and venous diameters were measured with a retinal vessel analyzer (RVA). Retinal blood velocity was assessed with bidirectional laser Doppler velocimetry. Thereafter, indomethacin (Confortid; Alpharma ApS, Copenhagen, Denmark) or placebo (NaCl 0.9%) was administered as an intravenous bolus dose of 0.4 mg/kg body weight over 5 minutes, followed by continuous infusion of 0.4 mg/kg/h over 2 hours. All hemodynamic measurements were repeated 0.5, 1, 2, and 3 hours after the start of the infusion. Intraocular pressure was measured using an applanation tonometer. Then subfoveal choroidal blood flow and fundus pulsation velocities were measured 0.5, 1, 2, and 3 hours after the start of the infusion.

**Methods**

**Noninvasive Measurement of Systemic Hemodynamics.** Systolic, diastolic, and mean arterial (MAP) pressure were measured every 10 minutes on the upper arm using an automated oscillometric device. Pulse rate was automatically recorded from a finger pulse-oxymetric device. An electrocardiogram was monitored continuously using a standard four-lead device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA).

**Retinal Vessel Analyzer.** The RVA (Imedos, Jena, Germany) is a commercially available system consisting of a fundus camera, a video camera, a high-resolution video recorder, a real-time monitor, and a personal computer with a vessel diameter analyzing software. The RVA allows for a precise determination of retinal vessel diameter with a time resolution of 25 readings per second. The fundus is illuminated with light in the range of wavelengths between 567 nm and 587 nm. In this spectral range, the contrast between retinal vessels and the surrounding tissue is optimal. Retinal irradiance was approximately 220 µW/cm², which is approximately 50 times lower than the maximum level allowed for constant illumination of the retina at the wavelengths mentioned. The system provides excellent reproducibility and sensitivity.13-14 In the present study, major temporal arteries and veins were studied. Measurements of retinal arterial and venous diameters were taken between 1 and 2 disc diameters from the margin of the optic disc.

**Laser Doppler Velocimetry.** In the present study, we used a fundus camera-based system with a single-mode laser diode at a centerline wavelength of 670 nm (Oculix 4000; Oculix Sarl, Arbaz, Switzerland). The principle of blood flow velocity measurement by laser Doppler velocimetry is based on the optical Doppler effect. Laser light, which is scattered by moving particles (e.g., erythrocytes), is shifted in frequency. This frequency shift is proportional to the blood flow velocity in the retinal vessel. The maximum Doppler shift corresponds to the centerline erythrocyte frequency. Measurements were made in the inferior temporal retinal veins.15 Red blood cell velocity was measured at the same locations as retinal vessel diameters. To obtain optimal readings, velocity data were taken from retinal veins.

**Calculation of Retinal Blood Flow.** Blood flow in retinal veins was calculated based on the measurements of maximum erythrocyte velocity ($\text{V}_{\text{max}}$) and retinal vessel diameters, both assessed in retinal veins at the same vessel location. Mean blood flow velocity was calculated as ($\text{V}_{\text{max}}/2$). Blood flow through a specific retinal vein was then calculated as $\text{Q} = (\text{V}_{\text{max}}/2) \times (\pi \times d^2/4)$, where $d$ is the diameter of the vein.

**Laser Doppler Flowmetry.** Measurements of subfoveal choroidal blood flow were performed by laser Doppler flowmetry (Oculix 4000; Oculix Sarl), introduced by Riva et al.16 For this purpose the vascularized tissue was illuminated by coherent laser light. Scattering on moving red blood cells (RBCs) leads to a frequency shift in the scattered light. In contrast, static scatterers in tissue do not change light frequency but lead to randomization of light direction impinging on RBCs. This light diffusing in vascularized tissue leads to a broadening of the spectrum of scattered light, from which mean RBC velocity (vel), blood volume (vol), and blood flow (flow) can be calculated in relative units. In the present study laser Doppler flowmetry was performed in the fovea to assess choroidal blood flow.
Table 1. Baseline Parameters on Indomethacin Day and Placebo Day

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<tr>
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<th>Indomethacin Day Baseline</th>
<th>Placebo Day Baseline</th>
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<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>81 ± 5</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>66 ± 12</td>
<td>64 ± 10</td>
</tr>
<tr>
<td>IOP (mm Hg)</td>
<td>13 ± 1</td>
<td>12 ± 2</td>
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<tr>
<td>Choroidal blood flow (au)</td>
<td>13.8 ± 3.3</td>
<td>11.5 ± 2.1</td>
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<tr>
<td>FPA (μm)</td>
<td>4.8 ± 1.3</td>
<td>4.8 ± 1.4</td>
</tr>
<tr>
<td>Retinal arterial diameter (μm)</td>
<td>125.7 ± 15.8</td>
<td>124.8 ± 15.9</td>
</tr>
<tr>
<td>Retinal venous diameter (μm)</td>
<td>151.7 ± 9.5</td>
<td>152.0 ± 6.6</td>
</tr>
<tr>
<td>Red blood cell velocity (cm/s)</td>
<td>2.0 ± 0.5</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>Retinal blood flow (μL/min)*</td>
<td>11.1 ± 2.4</td>
<td>10.8 ± 2.7</td>
</tr>
<tr>
<td>OPP (mm Hg)</td>
<td>40.9 ± 3.3</td>
<td>40.4 ± 4.5</td>
</tr>
<tr>
<td>Retinal vascular resistance (au)</td>
<td>2.4 ± 0.7</td>
<td>2.4 ± 0.6</td>
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Values are presented as mean ± SD.
* Retinal blood flow through one vein only, not total retinal blood flow.

Laser Interferometry. Pulse synchronous pulsations of the eye fundus were assessed by laser interferometry. The method is described in detail by Schmetterer et al.17 Briefly, the eye is illuminated by the beam of a single-mode laser diode (λ = 783 nm) along the optical axis. The laser power of not more than 100 μW is much lower than the limit set by the American National Standards Institute. The light is reflected at the front 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. The fundus pulsation amplitude (FPA) has been shown to estimate the pulsatile blood flow component in the choroid.18,19 FPA measurements were performed in the macula.

Measurement of Intraocular Pressure. IOP was measured with a Goldmann application tonometer (Haag-Streit, Vienna, Austria) before drug administration and at 1 hour and 3 hours after the start of infusion.

Calculation of Vascular Resistance. Ocular perfusion pressure (OPP) was calculated as 2/3MAP – IOP.20 In the present study, retinal blood flow was assessed in one vein only. Assuming that the results in this vein were representative for total retinal blood flow, retinal vascular resistance was calculated as OPP divided by retinal blood flow.

Statistical Analysis

For data description, hemodynamic parameters were expressed as percentage change from baseline (Δ%). Effects of indomethacin and placebo on hemodynamic parameters were assessed by two-way ANOVA for repeated measurements using the absolute values. Post hoc analysis was performed using planned comparisons. A two-tailed P < 0.05 was considered the level of significance. Results are given as mean ± SD. Calculations were performed using the Statistica software package (Statsoft, Tulsa, OK).

RESULTS

Systemic Hemodynamics and Intraocular Pressure

Baseline pulse rate and IOP were comparable on both study days (Table 1). Neither administration of indomethacin nor placebo affected MAP, pulse rate, OPP, or IOP.

Retinal Blood Flow Parameters

As shown in Figure 2, retinal arterial diameters decreased significantly after administration of indomethacin, reaching a maximal decrease of −4.3% ± 3.4% 180 minutes after start of the infusion (ANOVA, P < 0.05, treatment vs. placebo), whereas no significant change was observed in retinal veins (ANOVA, P = 0.08, treatment vs. placebo). Retinal blood cell velocity, taken from veins decreased by a maximum of −29% ± 20% (ANOVA, P < 0.05, treatment vs. placebo). Hence, calculated retinal blood flow also decreased up to −27% ± 21%, reaching the strongest effect 60 minutes after infusion start, an effect that was highly significant (Fig. 2; ANOVA, P < 0.05, treatment vs. placebo). Placebo did not affect any of the parameters measured. In the indomethacin group, calculated vascular resistance increased by 48% ± 36% and 26% ± 29% 60 minutes and 180 minutes after the start of drug administration, respectively. No change in vascular resistance was observed in the placebo group (data not shown).

Choroidal Blood Flow Parameters

Subfoveal choroidal blood flow decreased immediately after administration by −17% ± 13% (Fig. 2; ANOVA, P < 0.05, treatment vs. placebo), whereas no change was observed in the placebo group. FPA also decreased after the administration of indomethacin (−7% ± 4%, ANOVA, P < 0.05, treatment vs. placebo), an effect not seen with placebo.

DISCUSSION

Vascular effects of arachidonic acid metabolites are complex and dependent on the subtype and the vascular bed investigated. Among these metabolites, PGs play a major role in the control of vascular tone.1–3 Whereas the role of PGs in blood flow regulation in several organs, such as the heart or the kidney,5,21 has been identified in some detail, our understanding of the role of PGs in the regulation of ocular circulation is still poor.

The paucity of knowledge can be at least partially attributed to the fact that several subtypes of prostaglandins with different pharmacologic properties and hemodynamic effects exist. Whereas prostaglandin F2α, prostaglandin E2, and thromboxane exert vasoconstrictory effects in isolated bovine retinal arteries,8 the predominantly produced PGs in the retina seem to be vasodilators, in particular prostacyclin (PGI2), which is endogenously released by the vascular endothelium.22 Furthermore, the interpretation of the data is hampered by the fact that considerable species differences exist in regard to the physiologic and anatomic properties of the tissue.25

The results of the present study are the first to demonstrate that intravenous administration of indomethacin induces a pronounced decrease of retinal and choroidal blood flow in humans. These results are in keeping with several observations from other vascular beds that indicate a reduction in brain blood flow, renal blood flow, and mesenteric blood flow after...
administration of the COX inhibitor indomethacin.9,11 Evidence from animal studies investigating the ocular circulation also confirms our findings. Several studies reveal a pronounced vasoconstrictory effect of COX inhibition in isolated ocular vessels.24,25 In addition, decreased optic nerve head oxygen tension was reported after the administration of indomethacin, which was again attributed to decreased blood flow induced by COX inhibition.26

Based on these observations, one might argue that the vessels of the ocular circulation are in a state of dilatation caused by constant production of the predominantly dilatating PGs, as is known, for example, for nitric oxide. However, results from recent reports suggest that hemodynamic effects of indomethacin may not be entirely caused by the inhibition of COX.10,27,28 More specifically, it has been observed that the vasoconstrictory effects of indomethacin differ from those of other nonsteroidal anti-inflammatory drugs (NSAIDs). Whereas the blood flow–decreasing effect of indomethacin could be confirmed in several experiments, no effect was observed after administration of the NSAID ibuprofen.10,27,28 The reason for this effect has yet to be identified, but the indication is that mechanisms other than inhibition of COX may be involved. Whether this holds true also for the human retina remains, however, to be investigated.

FIGURE 2. Effect of indomethacin (A) or placebo (C) on the blood flow parameters. All data are presented as mean ± SD. #*P < 0.05 ANOVA between groups. *P < 0.05 versus baseline, planned comparisons, post hoc testing. Red blood cell velocity was taken from retinal veins.
One possible explanation for the different effects of indomethacin and other NSAIDs on ocular blood flow may be related to indomethacin-induced release of other vasoactive substances, most importantly nitric oxide (NO).\textsuperscript{29,30} Although the exact mechanism for the interaction between PG and NO is still unclear, PG and NO contribute together and independently in the control of circulation.\textsuperscript{31–33} Further studies are, however, required to clarify whether this plays a role in the indomethacin-induced ocular vasoconstrictor response.

Interestingly, we observed a decrease in retinal arterial diameters in the present study, whereas no change was observed in retinal veins. At the first glance, this seems contradictory. However, vascular tone in veins is mainly dependent on the upstream blood flow. In the retina, as in every other vascular bed, blood flow is controlled by the perfusion pressure and the vascular resistance to flow. Vascular resistance is regulated for the most part in the microcirculation, namely retinal resistance vessels with diameters smaller than 40 μm. Hence, vasoconstriction in the microcirculation in response to indomethacin may well contribute to the decrease in retinal blood flow. This hypothesis is supported by our data suggesting that calculated retinal vascular resistance increased 47% after drug administration. Given the law of Hagen-Poiseuille, which indicates the resistance to be inversely proportional to the radius raised to the fourth power, we would expect only a 16% increase in vascular resistance based on the observed constriction of the major retinal arteries. Thus, the constriction at the arterial site does not seem to fully account for the decrease in venous blood flow, and it is not unreasonable to hypothesize that a more pronounced constriction occurred in the smaller arterioles than in the arteries measured in this study. In addition, it is unknown whether indomethacin alters the diameter of the upstream blood vessels, namely the ophthalmic artery, so that the pressure in the central retinal artery is lower than expected. This, however, appears to be unlikely, because there is no evidence to show that indomethacin increases vascular tone in the ophthalmic artery. When discussing the results of our data in the choroid, a number of limitations must be considered. Laser Doppler flowmetry measurements are restricted to the subfoveal area. Whether the peripheral choroid also constricts in response to indomethacin is unclear. With FPA, however, only the pulsatile component of flow to the retina and choroid in the newborn pig after nonsteroidal antiinflammatory drugs. Invest Ophthalmol Vis Sci. 1992; 33(12):3378–3384.

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