Role of Adenosine in the Control of Choroidal Blood Flow during Changes in Ocular Perfusion Pressure

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PURPOSE. The purpose of the present study was to investigate whether the nucleoside adenosine is involved in the regulatory processes of choroidal blood flow (ChBF) during an experimental decrease in ocular perfusion pressure (OPP).

METHODS. In this randomized, double-masked, placebo-controlled, two-way crossover study, 14 subjects received either intravenous adenosine or placebo on two different study days. The suction cup method was used for a stepwise increase in intraocular pressure (IOP). Subfoveal ChBF was measured by laser Doppler flowmetry. Mean arterial pressure (MAP) and IOP were measured noninvasively. Ocular perfusion pressure was calculated as OPP = 2/3 MAP – IOP.

RESULTS. Adenosine increased ChBF significantly versus placebo before application of the suction cup (P < 0.05). When the suction cup was applied, a significant decrease in OPP was observed. This effect was comparable on all study days. The decrease in OPP was paralleled by a significant decrease in ChBF (maximum between −43% and −52%) which was less pronounced than the decrease in OPP (maximum between −62% and −64%). Neither placebo nor adenosine influenced the ChBF increase during suction cup-induced changes in OPP.

CONCLUSIONS. The data of the present study confirm that the human choroid shows some regulatory capacity during a decrease in OPP. Adenosine influences basal vascular tone in the choroid but is not involved in the regulatory mechanisms during an increase in IOP. (ClinicalTrials.gov number, NCT00712764.) (Invest Ophtalmol Vis Sci. 2011;52: 6035–6039) DOI:10.1167/iovs.11-7491

Autoregulation is defined as the ability of a vascular bed to maintain its blood flow despite changes in perfusion pressure. It is well documented that retinal blood flow is autoregulated in response to changes in perfusion pressure.1–6 In the past 20 years, evidence has accumulated that the choroid also shows some regulatory capacity.7–15 Most of these data come from experiments using laser Doppler flowmetry (LDF). Before that era, the choroid was assumed to be a strictly passive vascular bed.11–17 Choroidal blood flow (ChBF) seems to be better regulated in response to an experimental increase in mean arterial pressure (MAP) than an increase in intraocular pressure (IOP).11,18–22

The mechanisms behind ChBF regulation during changes in perfusion pressure have yet to be investigated. Since the pressure–flow relationship in the choroid appears to be unaltered during moderate hypercapnia and hyperoxia, a metabolic mechanism seems unlikely.18 The choroid, however, shows rich neuronal innervation, indicating that neurogenic mechanisms are involved in ChBF regulation.12 As such, blood flow regulation in the choroid cannot be considered autoregulation in its strict sense, because the term refers to an isolated vascular bed. A myogenic mechanism may also play a role in ChBF adaptation during changes in ocular perfusion pressure (OPP).7

The purine adenosine is a breakdown product of cellular adenosine triphosphate (ATP). P1 receptors, which are selective for adenosine, can further be divided into A1, A2A, A2B, and A3 receptors.23 In the eye, two of these receptors have been localized in the retina: A1 and A2B.24 In general, activation of adenosine receptors leads to changes in adenylyl cyclase activity, and whereas activation of A1 receptors results in attenuation of intracellular cyclic adenosine 3',5'-mono-phosphate (cAMP) levels and therefore in vasodilation, activation of A2B receptors is associated with elevation of intracellular cAMP levels leading to vasodilatation.25 The role of adenosine in the eye is still controversial. Adenosine receptor stimulation seems to protect the retina against ischemia–reperfusion damage.26 Further, there is evidence from several animal studies and recent human studies that adenosine causes choroidal and retinal vasodilatation.27–29 Gidday and Parks30 suggested that adenosine is a key participant in mediating regulatory adjustments in retinal blood flow. They demonstrated that arterioles of the newborn piglet retina dilate dose dependently in response to a pharmacologically induced increase in endogenous, interstitial adenosine concentration. Potentiation or inhibition of endogenous adenosine affects the retinal arteriolar dilatative response to hypoxia and hypotension.30 Adenosine also seems to control ocular blood flow in humans. In a dose–response study, adenosine induced significant effects on choroidal and optic nerve head blood flow.27 The aim of the present study was to investigate whether adenosine plays a role in ChBF regulation during a decrease in OPP.

MATERIAL AND METHODS

Subjects

The present study was performed in compliance with the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines of the European Union. The study protocol was approved by the Ethics Committee of the Medical University of Vienna. After written informed consent was obtained, 14 healthy male subjects participated (age, 26.3 ± 4.3 years, mean ± SD). The number of subjects was based on a

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sample size calculation using data from a previous study investigating the pressure-flow relationship during an increase in IOP measured with LDF.\textsuperscript{12} Sample size was calculated using a double-sided \( \alpha \) error of 0.05 and a \( \beta \) error of 0.20, to detect a 15% difference between the active drug and placebo. During the 4 weeks before the first study day, each subject had to pass a pretreatment screening that included recording of medical history and a physical examination. Twelve-lead electrocardiogram, complete blood count, activated partial thromboplastin time, thrombin time, clinical chemistry, urine analysis, and an ophthalmic examination. If any abnormality was found as part of the pretreatment screening the subject was not included unless the investigators considered an abnormality to be clinically irrelevant. In addition, only subjects with ametropia of less than 3 D and no evidence of eye disease that might interfere with the purpose of the study were recruited to participate in the trial. During the last week after completion of the study, a follow-up safety investigation was scheduled. This follow-up investigation was similar to the pretreatment examination.

**Drugs and Drug Administration**

The study drugs were administered by intravenous infusion. The following drugs and doses were administered: adenosine sodium chloride solution (3-mg/mL vials Ebewe Pharma GmbH; Unterach am Attersee, Austria; infusion period, 30 minutes; dosage, 40 \( \mu \)g/kg/min)\textsuperscript{27} and physiologic saline solution (as a control substance; infusion period 30 minutes).

**Methods**

**Noninvasive Measurement of Systemic Hemodynamics.** Systolic (SBP), diastolic (DBP), and MAP were measured on the upper arm with an automated oscillometric device. Pulse rate (PR) was automatically recorded with a finger pulse-oximetric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA).

**Laser Doppler Flowmetry.** Measurement of ChBF was performed by LDF\textsuperscript{31,32} with a compact instrument.\textsuperscript{33} For this purpose, the vascularized tissue is 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 directions impinging on RBCs. From the Doppler shift power spectrum, the mean RBC velocity is calculated in relative units. In the present study, LDF was performed in the fovea to assess ChBF.

**IOP, OPP, and Vascular Resistance.** A slit-lamp-mounted Goldmann applanation tonometer was used to measure IOP. Before each measurement, one drop of 0.4% benoxinate hydrochloride combined with 0.25% sodium fluorescein was used for local anesthesia of the cornea. OPP was calculated as \( \frac{3}{2}(\text{MAP} – \text{IOP}) \).\textsuperscript{6}

**Suction Cup Method.** IOP was increased using the episcleral suction-cup technique, which has been described previously by Ulrich and Ulrich.\textsuperscript{34} While the eye was topically anesthetized, a rigid, standardized, 11-mm diameter plastic suction cup was placed on the temporal sclera with the anterior edge at least 1 mm from the limbus. Through plastic tubing, the suction cup was connected to an automatic suction pump, which was used to increase the vacuum from 50 to 125 mm Hg to produce an IOP increase.

**Study Design**

The study was performed in a randomized, double-masked, placebo-controlled, two-way crossover design. Subjects were assigned to receive intravenous infusions of either adenosine or physiologic saline solution on two different study days. The minimum washout-period between the two study days was 4 days.

On the trial days, baseline measurements of ChBF and systemic hemodynamics were performed after a 20-minute resting period. Thereafter, the suction cup was applied with a suction of 50 mm Hg. The suction was increased in three consecutive steps to 75, 100, and 125 mm Hg. Each suction level was maintained for 2 minutes, and ChBF was measured continuously. The procedure was repeated after a 30-minute resting period. Again, each suction level was maintained for 2 minutes and, instead of ChBF, IOP was measured at each incremental step. Thereafter, another resting period of at least 30 minutes was scheduled. Afterward, adenosine or placebo was administered intravenously for 30 minutes. During the last 11 minutes of drug administration, measurement of ChBF with stepwise increase of IOP was performed again. During all procedures, systemic hemodynamic parameters were assessed every 2 minutes, and heart rate was monitored continuously.

**Data Analysis**

Since subjects showed unstable reference signals (direct current, DC) during the measurements, the polynomial correction approach was applied, which was first introduced by Gugleta et al.\textsuperscript{55} Briefly, the parameter “yield” was calculated as DC/gain. A regression model applying a third-order polynomial equation was used on the logarithmic values of yield and the respective LDF parameters, to calculate the corrected LDF data.

An ANOVA model for repeated measurements was used to analyze the data. Statistical significance was analyzed by studying the interaction between time and treatment. In addition, the effect of the drugs under study on basal ChBF was assessed. Data are presented as the mean \( \pm \) SD. \( P < 0.05 \) was set as the level of significance.

**Results**

Fourteen subjects completed the study according to the protocol. The baseline characteristics of the study population are presented in Table 1. There were no significant differences between the baseline values on the two study days. Application of the suction cup at different suction levels caused a significant increase in IOP (to average: \(+13 \pm 2, +17 \pm 3, +21 \pm 2, +25 \pm 3 \text{ mm Hg}, P < 0.001 \) versus baseline). This effect was comparable on all study days. Under basal conditions, neither adenosine nor placebo influenced IOP (\( P = 0.20 \) between groups). Likewise, arterial blood pressure did not change after administration of placebo (\( -1.9 \pm 6.7 \text{ mm Hg} \) or adenosine (\( -1.9 \pm 4.3 \text{ mm Hg}; P = 1.0 \) between groups). Placebo had no significant effect on ChBF compared with the baseline values. Adenosine induced a significant increase in ChBF before application of the suction cup (\(+15\% \pm 15\%, P = 0.00445, \text{ Fig. 1})

The effects of suction cup application on OPP are shown in Figure 2. A pronounced reduction in OPP was observed when suction was applied (\( P < 0.001 \) versus baseline). The experimentally induced changes in OPP were comparable during the pretreatment periods on all study days (\( P = 0.20 \) between groups). There was no change in the responding pattern of OPP to suction cup application during infusion of placebo or adenosine, compared with pretreatment values (\( P = 0.50 \) between groups).

The decrease in OPP during suction cup application was paralleled by a significant decrease in ChBF in the pretreatment periods (maximum reduction of ChBF between 41.8 and 42.3\%, \( P < 0.001 \) versus baseline, \( \text{Fig. 3})

<table>
<thead>
<tr>
<th>Table 1. Baseline Data on Each Study Day</th>
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<tr>
<td><strong>Placebo Day</strong></td>
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<td><strong>Adenosine Day</strong></td>
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<tr>
<td>MAP, mm Hg</td>
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<td>PR, beats per minute</td>
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<td>IOP, mm Hg</td>
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<td>OPP, mm Hg</td>
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<td>ChBF, arbitrary units</td>
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Mean \( \pm \) SD; \( n = 14 \).
changes in ChBF were observed during infusion of placebo (−43.1% at maximum; \( P < 0.001 \) versus baseline) and adenosine (−1.9% at maximum, \( P < 0.001 \) versus baseline).

**DISCUSSION**

The data from the present study indicate that adenosine is not involved in the regulatory mechanisms of the choroid during a decrease in OPP. However, the results are in concordance with previous experiments in humans showing that a decrease in OPP is paralleled by a decrease in ChBF, which is less pronounced than the decrease in OPP,\(^\text{10,11}\) indicating some degree of ChBF regulation in response to a decrease in OPP.

However, the decrease in ChBF was less pronounced than the decrease in OPP during all stimulation periods. There was no difference in the ChBF response on the two study days (\( P = 0.09 \) between groups).
This vasodilatation is probably mediated via A$_{2A}$ receptors, since enhanced intracellular cAMP levels relax vascular smooth muscle.\textsuperscript{56}

Although adenosine influences basal choroidal vascular tone, the findings of the present study suggest that it does not contribute to the regulatory mechanisms during an experimental decrease in OPP in humans since administration of adenosine did not alter the ChBF response during a decrease in OPP. In a study conducted in newborn piglets hemorrhagic hypotension was induced to lower OPP for investigation of retinal arteriolar blood flow regulation. Local interstitial adenosine potentiation significantly increased the dilative response to hemorrhagic hypotension. The authors therefore concluded that adenosine is a key participant in mediating regulatory adjustments in retinal blood flow.\textsuperscript{50} For the human choroid, this does not seem to be true.

Some limitations of the present study design in humans have to be mentioned. One problem is that subjects start at different baseline OPPs and that there is a wide variety in suction-cup-induced changes in IOP. In a crossover study, this problem is minimized, however, because each subject served as his own control and the ChBF response to changes in IOP shows good reproducibility. There are also some limitations in human studies of ChBF using LDF, which have been discussed in detail elsewhere.\textsuperscript{37} Briefly, this device measures only in the subfoveal choroid, and the depth of measurements is unknown. Therefore, the findings of the present study may not be applicable for peripheral parts of the choroid.

The use of an adenosine receptor antagonist would have been interesting because it would have given clearer insight into the role of adenosine in ChBF during an experimental decrease in OPP. However, to date there is no commercially available adenosine receptor antagonist for use in humans.

In conclusion, the data of the present study confirm that the human choroid shows some regulatory capacity during a decrease in OPP. Adenosine influences basal vascular tone in the choroid but is not involved in the regulatory mechanisms during an increase in IOP.

References


