Effect of histamine and cimetidine on ocular blood flow

Effect of histamine and cimetidine on retinal and choroidal blood flow in humans

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Abstract:

Intravenous administration of histamine causes an increase in choroidal blood flow and retinal vessel diameter in healthy subjects. The mechanism underlying this effect remains to be elucidated. In the present study we hypothesized that H₂ receptor blockade alters the hemodynamic effects of histamine in the choroid and the retina.

18 healthy male non-smoking volunteers were included (age range: 21-35 years, mean: 25.5 years, SD: 3.0) in this randomized, double masked, placebo-controlled two-way cross-over study. Histamine (0.32µg/kg/min over 30 minutes) was infused intravenously in the absence (NaCl as placebo) or presence of the H₂ blocker cimetidine (2.3mg/min over 50 min). Ocular hemodynamic parameters, blood pressure and intraocular pressure were measured before drug administration, after infusion of cimetidine or placebo and after co-infusion of histamine. 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. Retinal blood velocity was assessed with bi-directional laser Doppler velocimetry. Histamine increased subfoveal choroidal blood flow (+14±15%, p<0.001), fundus pulsation amplitude (+11±5%, p<0.001), retinal venous diameter (+3.0±3.6%, p=0.002) and retinal arterial diameter (+2.8±4.2%, p<0.01), but did not change retinal blood velocity. The H₂ antagonist cimetidine had no significant effect on ocular hemodynamic parameters. In addition, cimetidine did not modify the effects of histamine on choroidal blood flow (p=0.6 versus placebo), fundus pulsation amplitude (p=0.5 versus placebo), retinal venous diameter (p=0.2 versus placebo) and retinal arterial diameter (p=0.5 versus placebo).

The present data confirm that histamine increases choroidal blood flow and retinal vessel diameters in healthy subjects. This ocular vasodilator effect of histamine is, however, not altered by administration of a H₂ blocker. Whether the increase in blood flow is mediated via H₁ receptors or by other hitherto unidentified mechanisms remains to be elucidated.
Introduction

The importance of histamine as a regulator of vascular tone has been demonstrated for several vascular beds (6, 12). Based on these experiments, it has been hypothesized that histamine may also play a role in the regulation of ocular blood flow. This hypothesis is supported by several in vitro experiments indicating a vasodilatory role of histamine in the ocular circulation (7). Data of human experiments on the effect of histamine on ocular blood flow is, however, sparse.

We could recently demonstrate that intravenous administration of histamine causes an increased mean flow velocity (MFV) in the ophthalmic artery (18). This increase is paralleled by a vasodilatation of retinal vessels and an increase in choroidal blood flow (24). However, the mechanism underlying this effect is unclear, because different subtypes of histamine receptors exist and the receptor responsible for the ocular vasodilator effects of histamine in vivo has yet to be identified (3, 4, 8). Recent experiments in other vascular beds have identified both H₁ and H₂ receptors as possible candidates for vascular effects, but the H₂ type seems to be the dominant receptor participating in the vasodilator responses of histamine in many vascular beds (21).

Thus, the current study aimed to test the hypothesis that administration of cimetidine, a histamine type-2 receptor antagonist, is able to modify the blood flow response of intravenous histamine in the choroid and retina.
Material and Methods

Subjects

18 healthy male non-smoking volunteers were included (age range: 21-35 years, mean: 25.5 years, SD: 3.0 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 Vienna University School of Medicine and followed the guidelines of GCP 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), total IgE-antibodies, hepatitis A, B, C and HIV-serology, urine analysis, and a urine drug-screening. Only subjects with IgE plasma levels of less than 100kU/l were included. Subjects were excluded if any abnormality was found as part of the pretreatment screening unless the investigators considered the abnormality to be clinically irrelevant. Further exclusion criteria were history of migraine or other types of headaches. 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 and anisometropia of less than 1 diopter.

Study design

The dose of cimetidine was selected based on the results of a pilot study (n = 4; mean age: 26.1 years; age range: 22-31 years). In this open dose escalation study increasing doses of cimetidine of 0.3mg/min, 1mg/min, 1.6mg/min, 2.3mg/min were administered for 15 minutes each step. Systemic hemodynamic measurements and
blood flow measurements were performed using the same techniques as outlined for the main study at the end of each infusion step.

In the main experiment, subjects were studied in a randomized, double masked, two way crossover design with histamine in combination with the H₂ receptor antagonist cimetidine or placebo. Two study days were scheduled for each subject with washout periods of at least five days between study days. On both study days histamine was administered intravenously in a dose of 0.32µg/kg/min with a co-administration of either cimetidine in a dose of 2.3mg/min or placebo.

On the trial days subjects arrived after a light breakfast. Baseline hemodynamic parameters were recorded in a sitting position after the values had stabilized. Cimetidine (Cimetag® 200mg Ampullen, Smithkline Beecham Pharma, Brunn/Gebirge, Austria) or placebo was given intravenously over a period of 50 minutes. 5 minutes after the start of the infusion ocular hemodynamic parameters were assessed again in a predetermined order (fundus pulsation amplitude, laser Doppler velocimetry, laser Doppler flowmetry, retinal vessel analyzer). 20 minutes after the start of the cimetidine or placebo infusion histamine (Mayrhofer Pharmazeutika, Linz, Austria) was administered for 30 minutes. 15 minutes after the start of the infusion of histamine the measurement procedures were repeated again. Pulse rate and blood pressure were measured in 5 minute intervals and real time electrocardiogram was monitored continuously throughout the study period.

The dose of histamine was chosen based on our previous investigations on the effect of systemic nitric oxide synthase inhibition on histamine-induced headache and on ocular vascular effects after intravenous histamine administration (17, 24).
Methods

Noninvasive measurement of systemic hemodynamics
Systolic, diastolic and mean blood pressures (SBP, DBP, MAP) were measured every 5 minutes on the upper arm using an automated oscillometric device. Pulse pressure amplitude (PPA) was calculated as PPA=SBP-DBP. 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, USA).

Zeiss retinal vessel analyzer (RVA)
The RVA (Imedos, Jena, Germany) is a commercially available system which comprises a fundus camera (Zeiss FF 450, Jena, Germany), 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/second (9). The fundus was illuminated with light in the range of wavelengths between 567nm and 587nm. In this spectral range, the contrast between retinal vessels and the surrounding tissue is optimal. Retinal irradiance was approximately 220µW/cm², which is about 50 times lower than the maximum level allowed for constant illumination of the retina at the wavelengths mentioned above. The system provides excellent reproducibility and sensitivity (13). In the present study, major temporal arteries and veins were studied. Measurements of retinal venous diameters were taken between 1 and 2 disc diameters from the margin of the optic disc. Red blood cell velocity was measured at the same locations as retinal vessel diameters by using bi-directional laser Doppler velocimetry.

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 670nm (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. Because of the smaller vessel diameter of retinal arteries compared to retinal veins, aiming the laser beam on the vessel surface is more difficult in retinal arteries. Accordingly, measurements were done in a major inferior temporal retinal vein only(15).

Calculation of retinal blood flow

Retinal blood flow was calculated based on the measurements of maximum erythrocytes velocity (Vmax) using laser Doppler velocimetry and retinal vessel diameters using the RVA. Mean blood flow velocity in retinal veins was calculated as (Vmax/2). Blood flow through a specific retinal vein was then calculated as $Q=(V_{\text{max}}/2) \times (\pi \times d^2/4)$, where $d$ is the diameter of the vein.

Laser Doppler Flowmetry

Measurement of subfoveal choroidal blood flow was performed by laser Doppler flowmetry (Oculix 4000, Oculix Sarl, Arbaz, Switzerland) introduced by Riva et al. (14). 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 direction impinging on RBCs. This light diffusing in vascularized tissue leads to a broadening of the frequency spectrum of scattered light form which mean RBC velocity, the blood volume, and the blood flow can be calculated in relative
units. In the present study laser Doppler flowmetry was performed in the fovea to assess choroidal blood flow.

**Laser interferometry**

Pulse synchronous pulsations of the eye fundus were assessed by laser interferometry. The method is described in detail by Schmetterer and colleagues (18). Briefly, the eye is illuminated by the beam of a single mode laser diode (λ=783nm) along the optical axis. The laser power of not more than 100µW is much lower than the limit set by the American National Standards Institute. The light is reflected at both the front side 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 maximum distance change between cornea and retina is called fundus pulsation amplitude (FPA) and estimates the pulsatile blood flow component in the choroid (16, 19).

**Measurement of intraocular pressure (IOP)**

The IOP was measured with a Goldmann applanation tonometer.

**Statistical analysis**

For data description and statistical analysis hemodynamic parameters were expressed as percentage change from baseline (Δ%). Effects of histamine and cimetidine on hemodynamic parameters were assessed by a two-way ANOVA model for repeated measurements. Results are given as mean ±SD. Post-hoc analysis was performed using planned comparison. Shapiro-Wilks' W test was used to test for normal data distribution. Calculations were performed using the Statistica® software package (Statsoft, USA).
Results

Dose finding study

As depicted in figure 1, administration of cimetidine induced a dose dependent decrease in pulse rate (ANOVA, p=0.015). Other systemic and ocular hemodynamic parameters remained unaffected by administration of cimetidine (data not shown).

Main study

Baseline values of hemodynamic and ocular blood flow parameters were comparable between study days as summarized in Table 1.

Systemic hemodynamics and IOP

As shown in Figure 2, administration of cimetidine induced a significant decrease of pulse rate (ANOVA, p=0.021), which was not altered by co-administration of histamine. Administration of histamine tended to decrease MAP when placebo was co-administered (-4 ± 9%), but this effect was not different on the cimetidine study day (ANOVA, p = 0.17). PPA was not altered by either histamine or cimetidine administration. IOP remained unchanged during the whole study period on both study days (data not shown).

Choroidal blood flow parameters

Administration of cimetidine alone did not affect choroidal blood flow or FPA (Figure 2). Histamine increased FPA assessed with laser interferometry and choroidal blood flow as assessed with laser Doppler flowmetry by +11±5% and +14±15%, respectively (ANOVA, p<0.001 each). Cimetidine did not modify the effects of histamine on FPA or choroidal blood flow (FPA: p = 0.51; choroidal blood flow: p = 0.63).

Retinal blood flow parameters
Cimetidine had no effect on retinal arterial or venous diameters, retinal red blood cell velocity or retinal blood flow. Administration of histamine increased retinal arterial diameters and retinal venous diameters by +2.8±4.2% (ANOVA, p=0.009) and +3.0±3.6% (ANOVA, p=0.002), respectively. Red blood cell velocity in retinal veins tended to decrease after administration of histamine (-8.3±21.2%, p<0.13), but this effect was not significant. Calculated retinal blood flow remained unchanged after administration of histamine (ANOVA, p=0.6). None of these effects were modified by co-administration of cimetidine.
Discussion

Whereas in several vascular beds the importance of histamine as a local regulator of blood flow has generally been accepted, the effect of histamine on ocular blood flow is less clear. Previous in vivo experiments in animal models did not indicate for a role of histamine in ocular blood flow regulation (1, 5), but other investigators provided evidence that histamine may induce vasodilation in the posterior part of the eye (7, 24).

Direct evidence of a vasodilatory effect of histamine has been recognized in isolated bovine retinal arteries, where a concentration dependent vasodilator effect of histamine was observed. These results observed in isolated vessels have been confirmed in animal experiments, where a pronounced increase in retinal blood flow in the rat has been observed following intravitreal administration of histamine (10). Little is, however, known about the mechanism underlying the vasodilator effect of histamine in the eye. The effect of histamine on isolated vessels was mainly attributed to the activation of H₁ receptors, although activation of H₂ receptors appears to contribute to the histamine response as well (7). Neither H₁ nor H₂ antagonists completely abolished the vasodilator response to histamine, but combined treatment with both antagonists totally suppressed the relaxing effect of histamine (7). In addition, the vasodilatory effect of histamine was dependent on the endothelium, indicating for a role of nitric oxide in the signaling cascade (7). This is in keeping with a previous report from our group showing that the effects of histamine in the ophthalmic artery and the choroid were diminished by systemic nitric oxide-synthase inhibition, supporting the concept that the vasodilator actions of histamine in the eye at least partially depend on nitric oxide (17).

Our data indicate that in vivo H₂ receptors do only play a minor role in mitigating the hemodynamic effects of histamine, because neither the histamine-induced vasodilator effects in the larger retinal vessels nor the effects on choroidal blood flow parameters
were altered by cimetidine. Could these results be false negative? We can not entirely exclude that higher doses of cimetidine may affect blood flow in the eye. On the other hand cimetidine was administered in a hemodynamically active dose as evidenced from the well known effect of the drug to induce a decrease in pulse rate (11).

This result is somewhat unexpected, because in other vascular beds H₂ receptors do contribute to histamine-induced vascular effects. It is already known for a long time that the action of histamine on vascular smooth muscle may vary depending on the tissue and species. Furthermore, histamine responses are known to be location and concentration dependent. Even a segmental vessel heterogeneity of the response to histamine has been described explaining differences between in vitro and in vivo studies (22, 23).

The potential sources of histamine in the retina are still a matter of discussion. On the one hand histamine does not cross the blood-retinal barrier. On the other hand mast cells, another potential source of histamine, are not present in the retina (20). Hence, histamine is likely to originate from neurons. In particular, horizontal cells have been proposed to be a source of histamine in the retina (2). This indicates that histamine acts as a local neurotransmitter or neuromodulator in the retina and contributes to the blood flow regulation in the posterior pole of the eye.

In conclusion we found that cimetidine in the selected dose was not able to modify the blood flow response to histamine. It remains to be elucidated, whether the histamine induced changes in ocular blood flow are mediated via H1 receptors or by other hitherto unidentified mechanisms.
Acknowledgements

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References


Effect of histamine and cimetidine on ocular blood flow


Table 1

<table>
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<tr>
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<th>Histamine day</th>
<th>Cimetidine / Histamine day</th>
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<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>82±7.5</td>
<td>80±7.1</td>
</tr>
<tr>
<td>Pulse rate (bpm)</td>
<td>67±9.2</td>
<td>68±11.3</td>
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<tr>
<td>Intraocular Pressure (mmHg)</td>
<td>13.1±1.5</td>
<td>12.5±1.7</td>
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<tr>
<td>Choroidal blood flow (arbitrary units)</td>
<td>10.2±2.3</td>
<td>10.5±2.2</td>
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<tr>
<td>Arterial vessel diameter (µm)</td>
<td>120.8±14.4</td>
<td>122.4±15.3</td>
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<tr>
<td>Venous vessel diameter (µm)</td>
<td>144.9±19.1</td>
<td>144.7±17.1</td>
</tr>
<tr>
<td>Fundus pulsation amplitude (µm)</td>
<td>4.2±1.4</td>
<td>4.3±1.4</td>
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<tr>
<td>Retinal blood velocity (cm/s)</td>
<td>2.0±0.6</td>
<td>2.0±0.7</td>
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<tr>
<td>Retinal blood flow (µl/min)*</td>
<td>16.4±4.3</td>
<td>16.2±4.2</td>
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</table>

Table 1: Baseline parameters of ocular and systemic hemodynamic measurements on the two study days. Baseline parameters were obtained during the 10 minutes before infusion. The averaging period for haemodynamic measurements with laser Doppler flowmetry and laser Doppler velocimetry was 120 seconds. Results are presented as means ± SD (n=18).

* Retinal blood flow denotes blood flow through one specific vein and not total blood flow.
Table 2: Systemic hemodynamic parameters of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure amplitude (PPA) and pulse rate (bpm) flow as recorded at the three timepoints.

<table>
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<tr>
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<th>Histamine day</th>
<th>Cimetidine / Histamine day</th>
<th>baseline</th>
<th>placebo</th>
<th>histamine</th>
<th>baseline</th>
<th>cimetidine</th>
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<tr>
<td>SBP (mmHg)</td>
<td>120±7</td>
<td>117±9</td>
<td>114±8</td>
<td>117±7</td>
<td>113±6</td>
<td>113±7</td>
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<tr>
<td>DBP (mmHg)</td>
<td>62±9</td>
<td>61±7</td>
<td>58±8</td>
<td>61±9</td>
<td>58±6</td>
<td>58±6</td>
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<td>MAP (mmHg)</td>
<td>82±7</td>
<td>80±7</td>
<td>76±6</td>
<td>80±7</td>
<td>77±5</td>
<td>77±6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPA (mmHg)</td>
<td>58±9</td>
<td>57±8</td>
<td>57±8</td>
<td>56±10</td>
<td>55±7</td>
<td>55±8</td>
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<tr>
<td>PR (bpm)</td>
<td>66±11</td>
<td>66±7</td>
<td>64±9</td>
<td>70±10</td>
<td>65±9</td>
<td>66±8</td>
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</table>
Figure 1: Effects of increasing doses of cimetidine on pulse rate (Pilot study, n = 4; means ± SD).
Figure 2: Effects of histamine in the absence (placebo, open up triangles) or presence of cimetidine (solid rectangles) on the outcome parameters (Main study, n = 18; means ± SD). * indicate significant effects of histamine versus baseline, # indicate significant effects of cimetidine versus placebo.