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Impaired Endothelial Function of the Retinal Vasculature in Hypertensive Patients

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Background and Purpose—Arterial hypertension constitutes a central factor in the pathogenesis of stroke. We examined endothelial function of the retinal vasculature as a model of the cerebral circulation.

Methods—Thirty-eight young subjects (19 hypertensive and 19 normotensive) were treated with the AT₁-receptor blocker candesartan cilexetil and placebo, each over 7 days. Retinal capillary flow and blood flow velocity in the central retinal artery were assessed with scanning laser Doppler flowmetry and pulsed Doppler ultrasound, respectively. N^G-methyl-L-arginine (L-NMMA) was infused to inhibit nitric oxide (NO) synthesis. Diffuse luminance flicker was applied to stimulate NO release.

Results—In normotensive subjects, L-NMMA decreased retinal capillary flow by 8.2% ± 13% ($P < 0.05$) and flickering light increased mean blood flow velocity in the central retinal artery by 19% ± 29% ($P < 0.01$). In contrast, no significant change to these provocative tests was seen in hypertensive subjects. Treatment with candesartan cilexetil restored a normal pattern of reactivity in retinal capillaries (L-NMMA: decrease in perfusion by 10% ± 17%, $P < 0.05$) and the central retinal artery (flicker: increase in mean blood flow velocity by 42% ± 31%, $P < 0.001$) in hypertensive patients.

Conclusions—Endothelial function of the retinal vasculature is impaired in early essential hypertension but can be improved by AT₁-receptor blockade. (*Stroke*. 2004;35:1289-1293.)

Key Words: cerebrovascular circulation ■ endothelium ■ hypertension ■ angiotensins ■ nitric oxide

Arterial hypertension is the predominant risk factor for ischemic stroke.¹ Apart from the level of blood pressure, nonhemodynamic factors including the renin-angiotensin-aldosterone system contribute to the risk of stroke and to infarct size. In animal models of ischemic stroke, therapy with angiotensin II subtype 1 receptor (AT₁-receptor) blockers improved survival.² In accordance, treatment with the AT₁-receptor blocker losartan reduces the risk of stroke in patients with arterial hypertension.³

AT₁-receptor blockers improve endothelium-dependent vasodilation in patients with arterial hypertension.⁴ In humans, the reduction in blood flow in response to nitric oxide synthase inhibitors has been used as a surrogate for the contribution of nitric oxide to the maintenance of perfusion in various vascular beds, including the forearm and the renal circulation, by us and others.⁴⁻⁶

The retinal vasculature is morphologically and functionally related to the cerebral vessels because of the common origin from the internal carotid artery. Recently, noninvasive techniques to measure retinal perfusion in humans have been developed,^{7,8} making the retinal vascular a useful model to obtain insight into endothelial function of the cerebral circulation. We conducted the present study to test the hypotheses that endothelium-dependent vasodilation of the retinal vascu-

lature is already impaired in early essential hypertension and that it can be restored by AT₁-receptor blockade.

Subjects and Methods

Study Participants

Study participants were screened for normal or mildly elevated blood pressure according to World Health Organization recommendations. Inclusion criteria were male gender and age between 18 and 35 years. Exclusion criteria were renal, hepatic, and any cardiovascular disease other than mild-to-moderate arterial hypertension (defined as 140 mm Hg ≤ systolic blood pressure < 200 mm Hg and 90 mm Hg ≤ diastolic blood pressure < 110 mm Hg, or both); eye disease other than grade I hypertensive retinopathy; any evidence of secondary hypertension; and any current cardiovascular medication (including antihypertensive medication). Regular smokers were not included; however, 4 subjects sporadically smoked cigarettes (up to 5 per month; none of the participants was allowed to smoke on the day of or the day before the retinal examination) and 3 subjects stopped smoking at least 1 year ago. Written consent was obtained before study inclusion. Two participants withdrew their consent after double-blind study medication had been distributed. Thus, 38 subjects (19 normotensives and 19 hypertensives) were examined. Clinical characteristics are given in Table 1.

Study Design

The study protocol was approved by the Clinical Investigation Ethics Committee of the University of Erlangen-Nürnberg. After inclusion

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TABLE 1. Clinical Characteristics

	Normotensives n=19	Hypertensives n=19
Age (y)	27±4	28±4
Weight (kg)	78±8	80±11
Height (m)	1.83±0.06	1.81±0.08
Body surface area (m ²)	2.00±0.12	2.00±0.16
Body mass index (kg/m ²)	23.3±1.6	24.4±2.7
Systolic blood pressure (mm Hg)	126±10	146±9†
Mean arterial blood pressure (mm Hg)	95±7	112±5†
Diastolic blood pressure (mm Hg)	79±7	90±14*

*†Significant differences between normotensive and hypertensive subjects.

* $P<0.01$. † $P<0.001$.

into this double-blind, randomized, crossover trial, study medication was distributed according to the randomization list. Participants were advised to take 1 tablet of study medication (either 16 mg of candesartan cilexetil or placebo) each morning for 7 days. On day 7, measurements of retinal perfusion were performed during the afternoon between 2 and 4 PM. After a 2-week washout phase, the second course of randomized study medication (either placebo or 16 mg of candesartan cilexetil) had to be taken for 7 days. A second measurement of retinal perfusion was performed on day 7.

The placebo-controlled crossover design enabled us to take hemodynamic data from the placebo phase as baseline perfusion parameters for comparison between normotensive and hypertensive subjects. The examiner performing the measurement of retinal perfusion (J.H.) was blinded to the participants' blood pressure status.

Experimental Protocol for the Measurement of Retinal Perfusion

In the first test, blood flow velocity in the central retinal artery was measured in the supine position after 30 minutes of rest at baseline, during flicker light stimulation (10 Hz; Photo Stimulator 750, Siemens-Elcoma AB; recordings started 5 to 20 seconds after the start of luminance flicker), and 10 to 15 minutes after flicker light exposure to ensure that blood flow velocity has returned to baseline values. Flickering light increases retinal blood flow via a nitric oxide-dependent mechanism.^{8,9}

In the second test, baseline retinal capillary flow was measured in the sitting position. The nitric oxide synthase inhibitor, *N*^G-monomethyl-L-arginine (L-NMMA; 3 mg per kg of body weight; Clinalfa) was administered intravenously as a bolus infusion over 5 minutes. Immediately after L-NMMA infusion, retinal capillary flow was measured again.^{10,11}

Measurement of Retinal Capillary Flow

Retinal capillary blood flow was assessed using scanning laser Doppler flowmetry at 670 nm (Heidelberg Retina Flowmeter, Heidelberg Engineering) as previously described in detail.⁷ Briefly, the Doppler shift in a retinal sample of 2.56×0.64×0.30 mm was scanned within 2 seconds at a resolution of 256 points×64 lines×128 lines. The confocal technique of the device ensured that only the capillary flow of the superficial retinal layer of 300 μm was measured. Measurements were performed in the juxtapapillary area of the right eye, 2 to 3 mm temporally to the optic nerve; the mean from 3 measurements was taken. Analysis of perfusion images was performed offline with automatic full-field analysis (Figure 1; SLDF version 3.3, Heidelberg Engineering).⁷ This led to a perfusion map excluding vessels with a diameter >30 μm, without lines with saccades, and without pixels with inadequate reflectivity. The mean retinal capillary flow was calculated in the area of interest and expressed as arbitrary units.

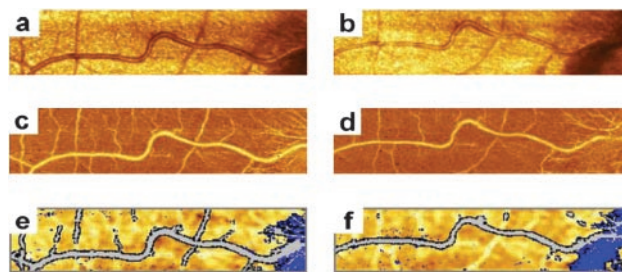


Figure 1. Assessment of retinal capillary perfusion by scanning laser Doppler flowmetry. a and b, Reflection images. c and d, Perfusion images. e and f, Perfusion images for automatic full-field analysis. Left images (a, c and e) were obtained at baseline, and right images (b, d and f) were obtained after infusion of L-NMMA. For better understanding, a larger arteriole is shown demonstrating the reduction in flow through L-NMMA (d vs c). For assessment of retinal capillary flow, such larger vessels are excluded for analysis.

Measurement of Blood Flow Velocity in the Central Retinal Artery

Blood flow velocity in the right central retinal artery was measured using pulsed Doppler sonography at 4 MHz (EME Companion, Nicolet Biomedical Inc) with a 10-mm Doppler probe as previously described.⁸ The mean measuring depth was 30±4 mm, with a gate (measuring distance) of 5 mm. Measurements were performed while the subjects' eyes were open. At least 10 pulse curves were averaged for quantitative analysis using an automated computer analysis (Flicker version 1.0, JT Harazny).

Statistical Analysis

All statistical analysis was performed using SPSS software (release 10.0, SPSS Inc). Significant deviations from normal distribution were excluded by the Kolmogorov-Smirnov test. Paired and unpaired Student *t* tests were used for comparisons when appropriate. A 2-tailed $P<0.05$ was considered to be significant. All values are expressed as mean±standard deviation.

Results

Systemic and Retinal Hemodynamics in Normotensive and Hypertensive Subjects

At baseline, in the placebo phase, blood pressure was significantly different between normotensive and hypertensive study participants (124±6/71±7 versus 137±11/81±7 mm Hg, $P<0.01$). In contrast, parameters for blood flow velocity in the central retinal artery and retinal capillary flow were similar across the groups (Table 2).

In normotensive subjects, L-NMMA significantly decreased retinal capillary flow already in the placebo phase by 8%±13% ($P<0.05$) (Figure 1). In contrast, L-NMMA had no significant effect on retinal capillary flow in hypertensive patients (Table 2). In normotensive subjects, L-NMMA increased mean arterial blood pressure by 3.9±3.7 mm Hg ($P<0.001$) and decreased heart rate by 11±6 minutes⁻¹ ($P<0.001$). In hypertensive subjects, L-NMMA decreased heart rate by 11±7 minutes⁻¹ ($P<0.001$) but had no significant effect on mean arterial blood pressure (increase by 1.3±8.9 mm Hg, $P=NS$).

In normotensive subjects, flickering light significantly increased blood flow velocity in the central retinal artery in the placebo phase by 21%±29% ($P<0.001$). In contrast, flickering light had no significant effect on blood flow

TABLE 2. Effects of Flickering Light and L-NMMA on Retinal Perfusion

	Normotensives n=19		Hypertensives n=19	
	Baseline	Flicker	Baseline	Flicker
Peak systolic blood flow velocity in the CRA (%)	11.3±1.8	12.7±2.5*	10.9±1.8	11.6±2.5
End-diastolic blood flow velocity in the CRA (%)	3.9±0.8	5.0±1.2†	4.4±1.1	4.8±1.3
Mean blood flow velocity in the CRA (%)	6.6±1.0	7.8±1.7†	6.9±1.3	7.3±2.0
	Baseline	L-NMMA	Baseline	L-NMMA
Retinal capillary perfusion (AU)	372±64	340±47*	365±77	373±88

L-NMMA indicates *N*^G-monomethyl-L-arginine; CRA, central retinal artery; AU, arbitrary units.

Data are given for the placebo phase (baseline values).

*†Significant changes through flickering light or L-NMMA. **P*<0.05. †*P*<0.01.

velocity in the central retinal artery in hypertensive patients (Table 2). Flicker light stimulation had no significant effect on blood pressure in normotensive and hypertensive subjects.

Effect of AT₁-Receptor Blockade on Systemic and Retinal Hemodynamics

At baseline, candesartan cilexetil significantly decreased systolic (*P*<0.05) and diastolic blood pressure (*P*<0.01) in hypertensive patients and diastolic blood pressure (*P*<0.01) in normotensive subjects (Table 3).

Candesartan cilexetil decreased mean blood flow velocity in the central retinal artery in the normotensive and hypertensive study participants (both *P*<0.05). Retinal capillary flow significantly decreased in normotensive subjects only (*P*<0.01) (Table 3).

In normotensive subjects, the response of retinal capillary flow to L-NMMA was not different between the placebo and the candesartan cilexetil phase. In contrast, the response of retinal capillary flow to L-NMMA was enhanced by candesartan cilexetil in hypertensive patients (from +3±17 to -10±17%; *P*<0.01) (Table 4, Figure 2).

Treatment with candesartan cilexetil did not change the response of blood flow velocity in the central retinal artery to flicker light stimulation in normotensive subjects. In contrast, treatment with candesartan cilexetil markedly increased the response of blood flow velocity in the central retinal artery to flicker light stimulation in hypertensive patients (mean blood flow velocity: from +5%±37% to +40%±31%; *P*<0.05), thereby restoring the physiological response pattern (Table 4).

Discussion

Improvement of Endothelial Function by Candesartan Cilexetil

In young patients with essential hypertension without any hypertension-related end-organ damage, we found that retinal perfusion parameters are similar to those in normotensive control subjects. With resting and casual blood pressures being greater in hypertensive than in normotensive subjects, this indicates an already increased retinal vascular resistance in our hypertensive subjects. In accordance, the blunted response of retinal capillary flow to L-NMMA in hypertensive subjects indicates a reduced contribution of nitric oxide to the maintenance of retinal perfusion. Our data in hypertensive patients are in accordance with data in patients with type 1 diabetes in whom a reduced response of choroidal circulation to L-NMMA has been observed.¹²

Therapy with the AT₁-receptor blocker candesartan cilexetil restored both the contribution of nitric oxide to the maintenance of retinal perfusion and nitric oxide-dependent vasodilation in the retinal vasculature of patients with arterial hypertension. This increase in nitric oxide bioavailability in hypertensive patients appears to outweigh a possible reduction in retinal perfusion caused by the blood pressure-lowering effect of candesartan cilexetil, because in contrast to normotensive subjects, retinal capillary flow did not decrease in the candesartan cilexetil phase in hypertensive patients.

An increase in nitric oxide bioavailability and restoration of impaired endothelium-dependent vasodilation through

TABLE 3. Effect of Candesartan Cilexetil on Systemic and Retinal Hemodynamics

	Normotensives n=19		Hypertensives n=19	
	Placebo	Candesartan Cilexetil	Placebo	Candesartan Cilexetil
Systolic blood pressure at rest (mm Hg)	124±6	122±10	137±11	132±8*
Mean arterial blood pressure at rest (mm Hg)	92±7	90±8	103±8	101±6
Diastolic blood pressure at rest (mm Hg)	71±7	67±9†	81±7	75±8†
Peak systolic blood flow velocity in the CRA (cm/s)	11.3±1.8	10.6±1.0	10.9±1.8	10.4±1.5
End-diastolic blood flow velocity in the CRA (cm/s)	3.9±0.8	3.8±0.6	4.4±1.1	3.8±0.6†
Mean blood flow velocity in the CRA (cm/s)	6.6±1.0	6.1±0.7*	6.9±1.3	6.2±1.0*
Retinal capillary flow (AU)	372±66	326±55†	365±77	397±84

*†Significant changes between the candesartan cilexetil and the placebo phase.

**P*<0.05. †*P*<0.01.

TABLE 4. Effect of Candesartan Cilixetil on the Response of Retinal Perfusion to Flickering Light and L-NMMA

	Normotensives n=19		Hypertensives n=19	
	Placebo	Candesartan Cilixetil	Placebo	Candesartan Cilixetil
Response to flickering light				
Change in peak systolic blood flow velocity in the CRA (%)	+14±27	+24±17	+7±32	+26±22
Change in end-diastolic blood flow velocity in the CRA (%)	+29±34	+32±17	+8±43	+50±39*
Change in mean blood flow velocity in the CRA (%)	+19±29	+29±17	+5±37	+42±31*
Response to L-NMMA				
Change in retinal capillary flow (%)	-7±13	-7±19	+3±17	-10±17†

*†Significant changes between the candesartan cilixetil and the placebo phase.

* $P < 0.05$. † $P < 0.01$.

AT₁-receptor blockers has already been found in various vessel beds, including the forearm, coronary, and renal circulation.^{4,13–15} Potential mechanisms include reduced formation of reactive oxygen species through blockade of the AT₁-receptor and increased nitric oxide synthesis through stimulation of AT₂-receptors by endogenous angiotensin II.^{16,17} However, the effects of AT₁-receptor blockade on the endothelium function of human retinal vasculature have not yet been examined, although regional disparities of nitric oxide bioavailability between vascular beds have been described.^{18–20}

Endothelial Function of the Human Cerebral Circulation

The main reason for this lack of knowledge about endothelial function of the human cerebral circulation so far has been the limited access for measurement. Alternatively, the retinal circulation constitutes an ideal model to examine the regulation of cerebral blood flow. According to previous experiments, nitric oxide plays an important role in the regulation of retinal^{21,22} and cerebral blood flow.^{23,24} Nitric oxide deficiency contributes to the enlarged cerebral infarct size in L-NMMA-treated rats.²⁵ Furthermore, the renin-angiotensin-aldosterone system substantially contributes to stroke devel-

opment and infarct size. In animal models of ischemic stroke, AT₁-receptor blockers reduce the volume of total and cortical infarcts, reduce the decrease in cerebral blood flow at the peripheral area of ischemia, and improve survival.^{2,26,27} In AT₁-receptor-deficient mice, the infarct size after experimentally induced cerebral ischemia is smaller than in wild-type mice.²⁸ The AT₁-receptor blocker candesartan cilixetil prevents against brain edema in stroke-prone spontaneously hypertensive rats.²⁹ In patients with arterial hypertension, AT₁-receptor blockade at similar blood pressure control was superior to beta-receptor blockade to prevent the incidence of stroke.³ Our present data point to a link between the renin-angiotensin-aldosterone system and endothelium-dependent vasodilation of the retinal and thereby the cerebral vasculature.

Limitations

Three limitations of our study have to be discussed. First, comparison of baseline perfusion parameters between normotensive and hypertensive subjects and between the placebo and verum phases might be influenced by intra-individual variability of retinal perfusion. However, the response of retinal perfusion to stimuli such as L-NMMA and flickering light is less likely influenced by this variability. Second, we do not know whether our findings will hold true in hypertensive patients with end-organ damage such as a history of stroke. Third, we are aware of the fact that our study was placebo-controlled and not controlled against another active treatment. Although data from other vasculatures demonstrate superiority of AT₁-receptor blockers in terms of improvement of endothelial function,^{4,13–15} we do not know whether other antihypertensive agents improve nitric oxide bioavailability and nitric oxide-dependent vasodilation of the retinal vasculature, too. However, taken together with experimental and clinical data, our findings provide a basis for the use of the AT₁-receptor blocker candesartan cilixetil to improve endothelium-dependent vasodilation of the retinal and probably of the cerebral vasculature in patients with arterial hypertension.

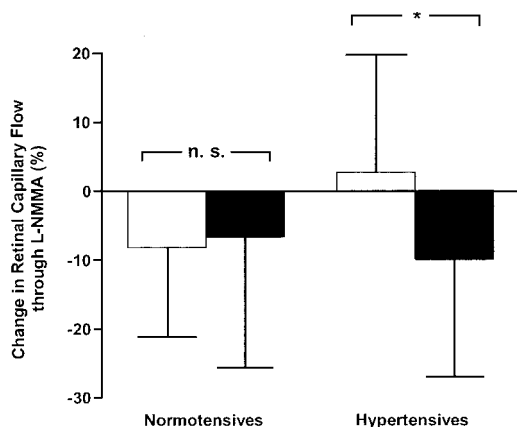


Figure 2. Effect of nitric oxide synthase inhibition on retinal capillary flow. Left columns (white) represent the placebo phase, and right columns (black) represent the candesartan cilixetil phase. NS indicates not significant. *Significant difference ($P < 0.05$).

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this investigator-initiated study, Takeda Pharma GmbH approved the final version of the study protocol but did not contribute to data analysis or to preparation of the manuscript. The contributions of Jeanette Kelm to this study are gratefully acknowledged.

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