Scanning Laser Doppler Flowmetry Shows Reduced Retinal Capillary Blood Flow in CADASIL

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Background and Purpose—Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a progressive systemic nonatherosclerotic angiopathy which causes ischemic strokes and vascular subcortical dementia. A cross-sectional study was performed to examine the retinal vascular caliber and blood flow in CADASIL.

Methods—Scanning laser Doppler flowmetry was used in a case–control study (11 patients and controls) of peripapillary retinal circulation. Automated full-field perfusion image analysis was used to analyze the flow data. Retinal vessel calibers were measured from retinal images acquired with scanning laser ophthalmoscopy. The caliber of the superior and inferior temporal retinal artery and vein were measured 1 and 2 mm from the disc rim, and the mean values were used for analysis.

Results—Retinal capillary peak systolic flow (mean, 249 versus 311 arbitrary unit [AU]; P=0.072) was lower, and mean capillary flow (mean, 184 versus 224 AU; P=0.12) and minimum diastolic flow (mean, 105 versus 132 AU; P=0.16) tended to be lower in patients than in controls. No significant difference in the calibers of proximal retinal arteries (mean, 104 versus108 μm) and veins (mean, 150 versus 145 μm) was found between the patients and controls.

Conclusions—Retinal capillary blood flow is mild to moderately reduced in CADASIL but that does not appear to cause major ischemic injury. Such reduction is analogous to that in the cerebral cortex in CADASIL patients with which retina appears to share its relative sparing from severe arterial ischemic tissue damage. (Stroke. 2004;35:2449-2452.)

Key Words: blood flow ■ CADASIL ■ ophthalmology ■ retinal vessels

In cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), both degeneration of vascular smooth muscle cells with deposition of granular osmiophilic material (GOM) in close proximity to them and secondary fibrosis can be found in small- and medium-sized arteries throughout the body as evidence of a systemic nonamyloid nonatherosclerotic arteriopathy.1,2 Although, the disease is generalized, neurological symptoms predominate. In 2 autopsied patients, GOM deposits were observed in retinal vessels (personal communication, Fritz H. Stefani, unpublished data, 1999).1 Arterial walls mainly appeared thickened but partly thinned. Vascular basement membranes were thickened, and loss of smooth muscle cells and fibrosis were observed. Furthermore, the pericytes of the central retinal artery were swollen, and focal demyelination of the optic nerve was observed.

Because the arteriopathy CADASIL also affects the retinal arteries, we evaluated retinal circulation in CADASIL by comparing vessel calibers and retinal blood flow of patients to those of age- and sex-matched controls.

Subjects and Methods

Eleven presymptomatic and symptomatic patients from 6 CADASIL families with R133C Notch3 mutation participated (10 females and 1 male; median age, 41 years; range 22 to 61). One patient had dementia, 4 others had a history of strokes, and 4 had only migraine. Three patients were presymptomatic. None of the patients had hypertension. Three patients were taking acetylsalicylic acid and 1 smoked cigarettes. None had eye diseases or any ocular medication. Eleven age- and sex-matched healthy controls (median age, 42 years; range 22 to 63; P=0.22, paired t test) were volunteers from the hospital staff. None took medications or smoked. The study was approved by the Institutional Review Board and all the subjects had given their informed consent.

Because the eyes of an individual are not independent of each other, only 1 eye of each participant was chosen for analysis at random. All cases and controls underwent a thorough clinical ophthalmologic examination including best corrected visual acuity.
Figure 1. Schematic representation of the retinal area used for scanning laser Doppler flowmetry, superimposed on HRT image of the optic disc. The white rectangles temporal to the disc (shaded white ellipse) show the 4 perfusion maps analyzed. Saccade artifacts (seen in map 3) were eliminated by recapturing or manual exclusion. Optic disc was excluded manually so that flow was measured from the nonshadowed area within the rectangles, automatically excluding larger blood vessels.

Intraocular pressure obtained with a Goldmann applanation tonometer, slit-lamp examination of the anterior segment, and dilated biomicroscopic fundus examination with the Volk 90 and 60 D lens and indirect ophthalmoscopy. Retinal vessel calibers were measured with scanning laser ophthalmoscopy (Heidelberg Retina Tomograph [HRT], Heidelberg Engineering; software 2.01). A 670 nm diode laser scanned a 2.8×2.8-mm field of the retina. Series of 2D images (resolution, 256×256 pixels) in 32 focal planes were acquired from superior and inferior temporal areas around the optic disc so that main retinal vessels were included. Two to 3 images from both areas were obtained, and the mean reflectivity image was calculated.

The caliber of the superior and inferior temporal retinal artery and vein were measured 1 and 2 mm from the disc rim. If the vessel branched before these points, the main branch was measured. Three replicate measurements from each point were averaged to obtain mean vessel caliber. Arteriovenous ratio was calculated by dividing the arterial caliber by the venous caliber at each point measured.

Retinal capillary blood flow was measured with scanning laser Doppler flowmetry (Heidelberg Retina Flowmeter [HRF], Heidelberg Engineering; software 1.02.4,5) The measurements were performed by 1 investigator (MH) who was not masked to whether a patient or control was examined. HRF generates a 2D flow map of erythrocytes in capillaries using a 2.8×0.7-mm field (64 horizontal lines). Maps were obtained that focused on the temporal peripapillary retinal nerve fiber layer around the optic disc at 4 locations (Figure 1).

Automated full-field perfusion image analyzer (Heidelberg Engineering, software 3.3), was used to calculate retinal perfusion from HRF images in arbitrary units (AU). Vessels wider than 30 μm, under- and overexposed pixels, and saccade artifacts were excluded as recommended. By computing the mean of each of the 64 lines and plotting results over time, mean, peak systolic (SF), and minimum diastolic (DF) capillary flow values were measured. Capillary pulsation index was calculated as $PI = 1 - (DF/SF)^{0.5}$ Mean values from the 4 areas (Figure 1) were used for analysis. One patient was excluded from automatic full-field perfusion image analyzer analysis because of insufficient quality of images.

Because of small sample size, $\alpha$ was set at 0.10 so as not to discard potentially important differences. Exact probability distributions were used (StatXact-3, Cytel). This study had 80% power to detect a difference of 10 μm in vessel caliber, 0.08 U in arteriovenous ratio, 53 AU in mean flow, 76 AU in peak arterial flow, 43 AU in minimum diastolic flow, and 0.072 U in capillary pulsation as statistically significant (Power and Precision 2.1, Biostat). Paired $t$ test and Pearson correlation coefficient were used, because all variables were normally distributed (Shapiro-Wilk test).

**Results**

All patients and controls had 20/20 best corrected vision. Intraocular pressure (13 versus 14 mm Hg; $P = 0.31$) and ocular perfusion pressure (79 versus 80 mm Hg; $P = 0.75$) in the examined eye of the patients and controls were comparable.

Upon clinical fundus examination by a retinal specialist (PS), the retinal arteries (the whole arterial tree evaluated) appeared narrow relative to age in all but 1 patient. However, when measured with the HRT, the calibers of retinal arteries of the CADASIL patients and controls (averaged measurements at a distance of 1 and 2 mm from the disc rim; mean, 104 versus 108 μm; $P = 0.40$ paired $t$ test) did not differ. The caliber of retinal veins (mean, 150 versus 145 μm; $P = 0.35$) and the arteriovenous ratio (mean, 0.70 versus 0.75; $P = 0.16$) were also of similar magnitude in the patients and controls (the differences in the values of the patients and controls are plotted in Figure 2A and 2B).

The caliber of arteries ($P = 0.84$ Pearson correlation) and veins ($P = 0.88$) of the CADASIL patients did not correlate with age, whereas in the controls the arterial caliber ($R = -0.65$; $P = 0.031$, Figure 2C) and arteriovenous ratio ($R = -0.762$, $P = 0.0052$; Figure 2D) correlated negatively with age.

Retinal capillary peak systolic flow (mean, 249 versus 311 AU; $P = 0.072$) was lower, and the mean capillary flow (mean, 184 versus 224 AU; $P = 0.12$) and minimum diastolic flow (mean, 105 versus 132 AU; $P = 0.16$) tended to be lower in the patients than in the controls (Figure 2E). Capillary pulsation indices ($P = 0.99$, Figure 2F) were comparable.

The mean capillary flow did not correlate with age in the patients ($R = 0.42$; $P = 0.23$) or in the controls (Figure 2G), but there tended to be a correlation with age as regards the peak capillary systolic flow in CADASIL patients ($R = 0.54$; $P = 0.11$; Figure 2H). No correlation with age in either group was found for minimum diastolic flow ($R = 0.16$; $P = 0.67$ in CADASIL) or capillary pulsation ($R = 0.17$; $P = 0.63$ in CADASIL).

No correlations between the arterial caliber versus capillary flow ($R = -0.13$; $P = 0.69$), versus peak systolic ($R = -0.26$; $P = 0.49$), versus minimum diastolic flow ($R = -0.45$; $P = 0.20$), and versus capillary pulsation ($R = 0.39$; $P = 0.28$) were found in the CADASIL patients.

**Discussion**

Our age- and sex-matched case–control analysis documents that CADASIL reduces retinal mean and peak systolic capillary flow. This agrees with the previously reported reduction of capillary flow on the optic nerve head rim measured with the HRF in CADASIL patients as well as with the irregular choroidal filling in retinal fluorescein angiography. CADASIL might also be a risk factor for nonarteritic anterior ischemic optic
neuropathy as recently reported, suggesting pathology of arteries supplying the optic nerve head.

Retinal vessels are transparent and their apparent caliber corresponds to the width of the blood column. Thickening of their walls would be seen as narrowing of the blood column. Our study did not have power to detect small differences in caliber, but it provides some evidence that narrowing of larger retinal arteries in CADASIL is likely to be compared with the normal population of similar age. The measurement in this study was done relatively proximally, ie, where the average diameters were m. On clinical examination, assessing also smaller retinal arterioles, the vessels appeared somewhat narrowed in all but 1 patient, similarly as reported by others. This agrees with postmortem findings that in the brain the short cortical arteries may even be dilated or are only slightly narrowed, whereas the long white matter penetrating arteries are markedly stenosed: in arteries with external diameters >100 μm, the profuse fibrosis may have reduced the internal diameters to smaller than 30 μm. This indicates that when examining the retina of CADASIL patients, emphasis should be placed on small caliber vessels. The retinal arterial caliber and arteriovenous ratio of the controls decreased with age and eventually resembled those of the patients. Aging thus masks the small difference present at younger age. It can be postulated that the lack of correlation between age and arteriovenous ratio in the CADASIL patients is a result of the ratio being already reduced in younger patients by the disease process.

The systolic and diastolic retinal flows were reduced proportionally so that pulsation indices were normal. Equally reduced systolic and diastolic flow in retinal capillaries is consistent with the cause being more proximal, ie, smooth muscle cell degeneration and fibrosis in the distal ophthalmic and central retinal artery and their branches (personal communication, Fritz H. Stefani, unpublished data, 1999). The retinal capillaries lack smooth muscle cells, the preferential site of damage in CADASIL, and, besides, they are smaller than those vessels known to be severely affected in CADASIL.

Figure 2. Difference in retinal vessel caliber (A) and arteriovenous ratio (B) between the CADASIL patients and controls (paired t test). Pearson correlation with age of arterial caliber (C) and arteriovenous ratio (D). Difference in mean and peak systolic retinal capillary blood flow as measured with scanning laser Doppler flowmetry (E) and in capillary pulsation index (F). Pearson correlation with age of the mean (G) and peak systolic (H) capillary flow. Note that capillary flow is reduced in CADASIL relative to controls (E), especially in younger age groups (G and H). In A, B, E, and F, every symbol corresponds to the difference between each case–control pair. A symbol below the dotted line at 0 indicates a smaller value for the CADASIL patient than for the control and vice versa. The shorter solid lines are the mean differences between all CADASIL patients and controls.
Unexpectedly, the retinal peak systolic flow values tended to increase with age in this small group of CADASIL patients, as opposed to the cortical blood flow in the brain, which decreased with age.\textsuperscript{14} Because CADASIL rarely causes death at the age of our patients, this finding is unlikely to be because of preferential survival of patients with better flow. Perhaps the only moderately affected retinal arteries can still react to more proximally impaired circulation.

Because our patients reported normal visual function and their visual acuity was normal, the impaired retinal blood flow does not appear to be of such severity that notable retinal damage had occurred. Correspondingly, in another group of 33 CADASIL patients, only a single retinal micro infarct and isolated chorioretinal scars as a sign of focal choroidal circulation insufficiency were noted on clinical examination (S.T., unpublished observation, 2004). This emphasizes the correspondence between the retina and cerebral cortex of CADASIL patients; the cerebral cortex is also spared from ischemic injury despite there being similar vascular smooth muscle cell degeneration and GOM deposition as in the white matter, where the lacunar infarcts are located (Miao et al, unpublished data, 2004). Longitudinal follow-up will show whether reduced retinal capillary blood flow is a prognostic sign of ocular complications in CADASIL.\textsuperscript{15}

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References
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