Impaired Endothelial Function of Forearm Resistance Arteries in CADASIL Patients

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Background and Purpose—Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary arteriopathy, which mainly involves the brain causing stroke and dementia. Mice expressing the mutated protein display early dysfunction in vasoreactivity in resistance arteries, but studies of patients have been inconclusive so far.

Methods—We examined peripheral endothelium-dependent vasodilatation in 10 CADASIL-patients and 20 controls using 3 methods: venous occlusion plethysmography of forearm blood flow with intraarterial acetylcholine and sodium nitroprusside infusions for evaluation of resistance arteries, ultrasound with flow mediated vasodilatation (FMD) of the brachial artery for evaluation of a conduit artery, and the pulse wave method with measurements before and after terbutaline for evaluation of systemic endothelium-dependent vasodilatation.

Results—The CADASIL patients displayed reductions in both basal (P=0.034) and stimulated blood flow (P=0.023 for the highest dose of acetylcholine) and an impaired endothelium-dependent vasodilation when investigated in forearm resistance arteries (P=0.019). The FMD and the pulse wave method did not show any reduction in endothelium-dependent vasodilatation in the patients.

Conclusions—Endothelium-dependent vasodilation was impaired in resistance arteries, but not in a conduit artery, in the forearm of CADASIL patients. (Stroke. 2007;38:2692-2697.)

Key Words: CADASIL syndrome ■ endothelium ■ vasodilation

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is caused by mutations in the NOTCH3-gene on chromosome 19p 13.2 to 13.1,1 which in adult humans is mainly or exclusively expressed in vascular smooth muscle cells (VSMCs).2 On histological examination, a generalized degeneration of VSMCs is seen mainly affecting small and medium sized arteries.3 However, VSMCs in arterioles in cerebral white matter are markedly more severely affected than those of the cortical arterioles4 and dermal arterioles are only mildly affected.5 Endothelial cells also display ultrastructural changes, depending on the different vascular beds.6

The main symptoms of CADASIL are cerebral: recurrent strokes, migrainous headache, psychiatric disturbances, and cognitive impairment.7 Corresponding to this clinical picture, imaging studies have shown that already in asymptomatic patients, the cerebral blood flow to the territories supplied by the affected arteries is reduced, especially in the white matter of the brain where most lacunar infarcts appear as the disease progresses.8,9 The age at onset and course of the disease are highly variable.7,10-12 Among the classical cardiovascular risk factors, only smoking has been reported to increase the risk of stroke in CADASIL.12 Although hypertension is not very common in CADASIL,12 cerebral microhemorrhages are frequent.13 Dysfunction in vasoreactivity has been suggested as an early event in the pathogenesis of CADASIL.14 A transgenic mouse model of CADASIL displays early in life an increased pressure-induced tone but decreased flow-induced vasodilation in resistance arteries15; however these results are difficult to interpret, because the mice studied so far do not develop a CADASIL-phenotype.16 Human arterioles studied ex vivo display an accentuated reaction to vasoconstricting substances, but normal response to vasodilatory agents.17

Because ischemia is the major problem in CADASIL, and the endothelium has a fundamental role in the regulation of blood flow, the previously described ultrastructural changes of endothelial cells might be relevant to the pathological process. We decided to evaluate endothelium-dependent vasodilatation both in conduit and resistance arteries together with measurements of blood flow, arterial compliance, and left ventricular function. The hypothesis was that endotheli-
um-dependent vasodilation would be impaired mainly in resistance arteries.

Materials and Methods

Subjects
Ten CADASIL-patients (6 men and 4 women, mean age 50.6 years, range 23.5 to 70) and 20 age- and sex-matched, healthy, nonsmoking control subjects without regular medication were investigated. One patient suffered also from type 1 diabetes mellitus, and another patient had a substituted hypothyroidism. The 10 patients came from 7 families. Their NOTCH3 mutations were R133C (n/H110051), R169C (n/H110053), C174R (n/H110051), R182C (n/H110051), C251Y (n/H110052), C435R (n/H110051). The mutation has not been identified for 1 patient, but the diagnosis was confirmed by unequivocal presence of granular osmiophilic material in the skin biopsy. Furthermore, this patient’s sister had characteristic CADASIL brain pathology at autopsy and autosomal dominant inheritance has been verified in the family. Two of the patients were monozygotic twins (with a previously unpublished C251Y mutation, Mykkänen et al, 2007), one of whom was asymptomatic at the time of this examination. The clinical symptoms of the 9 symptomatic patients were stroke (n/H110057), TIA (n/H110056), depression (n=2), acute encephalopathy (n=1), migraineous headache (n=3), and cognitive impairment (n=3). One of the patients was a current smoker. Five patients had treatment with low-dose aspirin, and 2 patients were treated with statins. The regional ethical committees approved the study and the subjects gave their informed consent.

Investigations
All the methods have previously been described in detail, including reproducibility.18,19 Patients and controls were investigated in the morning or at midday after fasting for more than 4 hours. Any medication, except insulin, was withheld on the day of examination. An arterial cannula was inserted into the brachial artery. Blood samples were taken for cell count and analysis of plasma lipids and glucose. Blood pressure was measured in the supine position after >20 minutes of rest both invasively in the cannulated arm and non-invasively (with a mercury sphygmomanometer) in the contralateral arm.

The Invasive Forearm Technique
Forearm blood flow was measured by venous occlusion plethysmography at baseline and during infusion of acetylcholine (a) and sodium nitroprusside (b). Mean values ± SEM. *P<0.05. Note the different scale on the y axis for the a and b panels.

The Brachial Artery Ultrasound Technique
The brachial artery was assessed by external B-mode ultrasound imaging 2 to 3 cm above the elbow (Acumen XP128 with a 10 MHz
linear transducer, Acumen). A blood flow increase was induced by inflation of a pneumatic cuff placed around the forearm to a pressure at least 50 mm Hg above systolic blood pressure for 5 minutes. Flow-mediated vasodilation was defined as the maximal brachial artery diameter recorded between 30 and 90 seconds after cuff release minus diameter at rest, divided by the diameter at rest. Blood flow velocity in the brachial artery was measured by Doppler before cuff occlusion and immediately after cuff release.

**Pulse Wave Analysis for Arterial Compliance and Vasoreactivity**

The pulse wave in the radial artery was captured by aplanation tonometry (Sphygmocor, Pulse Wave Medical Ltd). Central blood pressure values were obtained from a validated transfer function obtained by the manufacturer, and aortic augmentation index is calculated as the ratio between amplitude of the first reflected wave divided by the amplitude of the first systolic peak in the central pulse wave curve. The relative height of the first diastolic reflected wave (in the radial pulse wave curve), the reflection index, was used for evaluation of vasodilation (see instructive figure at http://avb.ahajournals.org/cgi/content/full/25/11/2368). After a baseline recording, terbutaline (0.25 mg subcutaneously) was given and a reevaluation was performed after 15 and 20 minutes, respectively. The maximal change of the reflection index following terbutaline in relation to the baseline was used.

**Carotid Artery Compliance and Intima-Media Thickness**

The diameter of the common carotid artery of the right side, 1 to 2 cm proximal of the bifurcation, was measured at its maximal diameter in systole and at its minimal diameter in diastole. The distensibility of the common carotid artery was calculated as the change in diameter maximum to minimum in relation to the minimal diameter in diastole divided by the central pulse pressure obtained by pulse wave analysis. Intima-media thickness of the common carotid artery was measured in the far wall 1 to 2 cm proximal of the bifurcation on both sides, and the mean value of those was used.

**Echocardiography and Doppler**

A comprehensive 2-dimensional and Doppler echocardiography was performed with an Acuson XP124 cardiac ultrasound unit (Acuson) with a 2.5 MHz transducer. Left ventricular diameters were measured with M-mode online from the parasternal projections, using leading edge to leading edge convention. Measurements included left atrial diameter, interventricular septal thickness, posterior wall thickness, and left ventricular diameter in end diastole and end systole. The left ventricular diastolic filling pattern of the mitral inflow was obtained from the apical transducer position with the pulsed Doppler sample volume between the tips of the mitral leaflets during diastole. The peak velocity of the early rapid filling wave (E wave) and the peak velocity of atrial filling (A wave) were recorded and the E to A ratio (E/A) was calculated. Left ventricular isovolumetric relaxation time was measured as the time between aortic valve closure and the start of mitral flow using the Doppler signal from the area between the left ventricular outflow tract and mitral flow. Left ventricular volumes were calculated according to Teichholz formula (7*D3/2.4+D) and from that stroke volume and ejection fraction were calculated. Analyses of the measurements from the ultrasound and plethysmography recordings were made by staff members blinded to whether a patient or a control person was examined.

**Statistics**

Differences between the groups in mean values for the different variables were evaluated by factorial ANOVA. Values for fasting glucose and E/A-ratio were log-transformed before evaluation, because they are known to be nonnormally distributed. Two-tailed significance values were given, with P<0.05 regarded as significant. The statistical program package Stat View (SAS Inc) was used.

**Results**

**Basic Characteristics**

There were no significant differences between the patients and controls regarding serum cholesterol or fasting blood glucose, but the patients had a higher body mass index than the controls. The diameters of the carotid and brachial arteries and of the left atrium and ventricle as well as intima media thickness of the common carotid artery did not differ between the patients and controls. The indices of left ventricular systolic (ejection fraction, cardiac index), and diastolic (E/A-ratio, isovolumetric relaxation time) functions were similar. Manually measured systolic and diastolic blood pressure did not differ significantly between the groups, but intraarterially measured pulse pressure was lower in the patients than the controls (51±7 SD versus 58±8 mm Hg, P=0.04.) Central pulse pressure was not significantly lower in patients. None of the indices of arterial compliance (carotid artery distensibility, stroke volume/central pulse pressure ratio, and aortic augmentation index) differed between the groups. For details, see Table 1.

**Flow-Mediated Dilatation of the Brachial Artery**

Flow-mediated dilatation of the brachial artery was similar in patients and controls. However, maximal blood flow velocity in the brachial artery was reduced both at baseline and during hyperemia in the CADASIL-patients (Table 2).

**Endothelium-Dependent Vasodilatation in Forearm Resistance Arteries**

Forearm blood flow measured by venous occlusive plethysmography was significantly reduced in the CADASIL-patients, both at rest (P=0.03) and after stimulation with acetylcholine in the high dose (Figure 1, P=0.02). The endothelial function index was lower in the patients than in the controls (P=0.019, Figure 2). The change in reflectance index from pulse wave analysis was not different between patients and controls (Table 2).

**Discussion**

The cardiac and large artery parameters did not disclose any significant differences between the patients and controls. Vasodilation of a conduit vessel, the brachial artery, was similar in patients and controls, which is also in agreement with a previous study. However, the present study showed a reduction in both resting and hyperemic forearm blood flow in the forearm of CADASIL patients compared with the controls. This difference in hyperemic blood flow was only significant for the highest dose of acetylcholine. Vasodilation of resistance vessels in response to infusions of nitroprusside was not significantly different, which agrees with the response of isolated resistance arteries to endothelium-independent vasodilator spermine-NONOate in vitro. However, the small number of patients, and the trend for reduced response, makes this conclusion uncertain.

To further evaluate the endothelial vasodilatory function in this setting, we used the endothelial function index, relating endothelium-dependent vasodilation to endothelium-independent vasodilation. We have previously used the en-
dothelial function index in a number of studies and found it to be correlated to the main cardiovascular risk factors, such as blood pressure, smoking, HDL-cholesterol, and a high-fat diet. This index has also been used by others who found a correlation with the major risk factors. The reduced endothelial function index in the forearm resistance vessels of CADASIL-patients indicates an impairment in endothelium-mediated vasodilation which cannot be explained only by structural (fibrosis and thickening) changes, ie, impaired distensibility, in the small arteries. Furthermore, decreased vasodilatation in the resistance vessels is also indicated by the reduced blood flow velocity seen during hyperemia in the

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SNP indicates sodium nitroprusside; Ach, Acetylcholine.
forearm of CADASIL patients. This agrees with findings in transgenic mice expressing mutant NOTCH3 which show early impairment in mechanotransduction, including flow-mediated vasodilatation in resistance vessels.\textsuperscript{15}

The pattern seen in the present study has some similarities to the findings in the cerebral circulation of CADASIL patients. Reduced basal and stimulated blood flow were recorded in affected white matter area in a study using the MRI bolus tracking method with acetazolamide as the vasodilator stimulus.\textsuperscript{8} A reduced basal cerebral blood flow, but preserved capacity for arterial acetazolamide-stimulated dilatation, was also demonstrated using phase-contrast MRI.\textsuperscript{9} A study with transcranial Doppler of the middle cerebral artery showed reduced CO\textsubscript{2} reactivity in CADASIL patients, a reduction that was more severe in demented CADASIL patients.\textsuperscript{14} Another transcranial Doppler study showed only reduced basal blood flow velocity, but preserved increase in response to CO\textsubscript{2} and to blood pressure reduction.\textsuperscript{26} A reduction of basal blood flow in CADASIL patients has also been described in cerebral white matter with PET\textsuperscript{27} and in retinal vessels.\textsuperscript{28}

The reduced endothelial function in smaller resistance arteries that we showed in this study may be a key element in the development of ischemic lesions in CADASIL patients especially in the brain, which is the organ with the highest degree of autoregulation of blood flow. The phenomenon of flow-mediated vasodilatation of large arteries is normally greater in the cerebral vasculature than in other vascular beds, increasing the risk of "steal" of blood flow from one vascular territory to another.\textsuperscript{29} Flow-mediated vasodilatation of vessels "downstream" is necessary to avoid "steal" in situations with increased blood flow and dilatation of large arteries.\textsuperscript{30}

Partial failure of vasodilatation in resistance arteries might occur early in CADASIL. Furthermore, the lack of collaterals makes the subcortical area vulnerable to the "steal" effect. A "steal" of blood flow from subcortical to cortical and extracranial vessels could hypothetically be the explanation for the subcortical location of the arteriopathy and ischemic lesions in CADASIL. Consistent with the "steal" hypothesis, an increased cortical blood flow, but normal or reduced blood flow in the white matter, was demonstrated in young CADASIL patients.\textsuperscript{27}

In CADASIL, arterial walls in the cerebral white matter and basal ganglia—the 2 main locations of infarcts—display not only a reduced lumen, but a marked thickening and fibrosis,\textsuperscript{4,31} already at an early stage of the disease. Such degenerative alterations might prevent normal flow-mediated vasodilatation in the affected arteries. The reduction of pulse pressure seen in our patients compared with controls agrees with another study showing a reduced systolic blood pressure in CADASIL patients.\textsuperscript{32} The degree of reduction in daytime mean arterial blood pressure is also correlated with the degree of cognitive decline.\textsuperscript{32} Such pulse pressure reduction might have indicated a reduction in cardiac output, contributing to a low basal blood flow in skeletal muscle and cerebral white matter, but our study did not reveal any significant reduction in cardiac performance. Secondly, it could be because of altered arterial compliance in conduit arteries, measured in the present study using 3 methods, but was normal in our patients. All these findings are consistent with the view that the basic disturbance in this patient group resides in the small arteries. This study also indicates that even though the basic fault is to be found in the VSMCs of the small arteries, the reduced vasoreactivity, possibly an important early event in CADASIL pathogenesis, may partly be caused by a defective function of the endothelial cells.

Because this is a rare patient group, the sample size is small and thereby the power to detect significant differences between patients and controls is limited. It should therefore be emphasized that only major impairments in the evaluated variables could be detected and that false-negative results could be produced. Thus, a lack of significance for a difference must be taken with caution in the present study. A strength of the study is the comprehensive investigation of the cardiovascular system, performed with multiple methods, to assess vascular function in different types of vessels.

In conclusion, this study showed normal flow-mediated vasodilatation in a conduit artery, but reduction in basal blood flow and indications of a reduced endothelial function of forearm resistance arteries, in CADASIL patients. If the endothelial function is also impaired in the resistance arteries in the brain, but not the conduit arteries, it may interfere with the normal distribution of cerebral blood flow and contribute to the development of subcortical vascular changes and lacunar infarcts in CADASIL patients.

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References


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