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Impairment of Endothelial Function in Patients with Spontaneous Cervical Artery Dissection: Evidence for a General Arterial Wall Disease

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Key Words
Carotid arteries · Vertebral arteries · Dissection · Endothelial dysfunction · Vascular risk factor

Abstract
Background and Purpose: Cervical artery dissection (CAD) accounts for 10–20% of ischemic strokes in young adults. Although trauma and preexisting disorders of the arterial wall are the main predisposing factors, most CADs are considered 'spontaneous'. We hypothesized that CAD could originate in systemic vascular disease bound to the intima–media interface without clinical signs. If this hypothesis is true, endothelium-dependent vasodilation would be impaired in response to a physiological stimulus such as an increase in blood flow. ***Methods:*** Flow-mediated arterial dilation was studied in 65 consecutive patients with spontaneous CAD: 26 with carotid artery dissection (ICAD), and 39 with vertebral artery dissection (VAD). CAD patients with vascular risk factors, trivial or obvious cervical trauma, or connective tissue disease were excluded. Twenty-three patients with ischemic stroke of unknown cause were included as controls. Using high-resolution ultrasonography, brachial artery diameter was measured at rest, during post-ischemic hyperemia (flow-mediated endothelium-dependent dilation), and after sublingual glyceryl trinitrate spray (endothelium-independent dilation). ***Results:*** The mean ± SD values of the flow-mediated vasodilation index were 5.7 ± 6.2% in ICAD, 5.0 ± 9.3% in VAD and 13.2 ± 6.5% in controls ($p < 0.0005$), without any difference between ICAD and VAD. Endothelium-independent dilation mean values were 21.5 ± 9.5% in ICAD, 25.1 ± 12.5% in VAD, and 20.8 ± 8.4% in controls, without a significant difference between groups ($p = 0.49$). ***Conclusions:*** These results give evidence of impaired endothelium-dependent vasodilation in CAD patients that is not the result of stroke, and suggest that an underlying abnormality of the arterial wall layers may predispose to CAD.

Cervical artery dissection (CAD) accounts for 10–20% of ischemic strokes in young adults [1–3]. Preexisting non-atheromatous intrinsic disorders of the arterial wall are supposed to be the main predisposing factors, while a cervical trauma can be a triggering factor for CAD [4, 5]. This condition, however, is absent in many CADs, leading to the concept of 'spontaneous' CAD [6, 7]. Several observations, especially the presence of concomitant dissections of cervical and renal arteries [8], an increased diameter of the aortic arch in patients with spontaneous CAD [9] and modifications of carotid wall properties [10], support the hypothesis that an asymptomatic general vascular disorder of the arterial wall may predispose to CAD.

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Among its multiple functions, the endothelium is normally responsible for a powerful local active vasodilatation [11, 12] that can be induced by physiological stimulations such as blood flow. Indeed, in physiological conditions most vessels react to an increased blood flow and increased shear stress by an increase in diameter [13], and it has been demonstrated that the so-called flow-mediated vasodilatation (FMD) is endothelium-dependent [12] and mainly due to nitric oxide (NO) release by endothelial cells [14]. The mechanism of NO production is initiated by the effect of shear stress on the endothelial cell [15].

This property is impaired in patients with vascular risk factors like smoking [16, 17], arterial hypertension [18], or hypercholesterolemia [16, 19]. Moreover, impairment of endothelium function occurs a long time before the appearance of clinical signs, suggesting that early subtle damage to the arterial wall may induce significant changes in endothelial response. Therefore, it can be expected that mild endothelial impairment, whatever its origin, could be revealed by examining the efficiency of a blood flow change on vessel diameter change. The aim of this study was to test the hypothesis that endothelial dysfunction is present in patients with spontaneous CAD.

Methods

All patients and controls gave written informed consent, and the study was approved by the local ethics committee.

CAD Patients

The recruitment of the patients and controls was realized in our stroke unit (Lille University Stroke Unit) where 1,200 stroke patients are seen every year. This department usually admits two types of stroke patients: those living in the city of Lille (1,177,396 inhabitants) and admitted directly as emergency patients, and those referred from other hospitals of the region (3,982,988 inhabitants) after a short stay in a neurological, medicine or emergency department. Patients of both types were included in the study provided they were hospitalized. Sixty-five patients aged 26–62 years with evidence of CAD were included consecutively between January 1998 and August 1999. Criteria for CAD were an enlarged arterial wall due to a hematoma on cervical ultrasonography or magnetic resonance imaging (T1-weighted MR axial images with fat saturation), or by a double lumen or intimal flaps on angiography. None of the CAD patients had any clinical abnormality suggesting a heritable connective tissue disorder, or trivial or obvious cervical trauma. All CAD patients in this study had had ischemic stroke. Due to our recruitment, we did not include patients with CAD without ischemic stroke. Twenty-six patients (mean age 41.8 ± (SD) 8.1 years; 9 women) had carotid artery dissection (ICAD) with 10 occlusion and 16 stenosis ICAD types. Thirty-nine patients (mean age 45.6 ± 7.9 years; 22 women) had vertebral artery dissection (VAD) with 8 occlusion and 11 stenosis VAD types.

Controls

As cerebral ischemia may have been a confounding factor, 23 patients (12 females) with ischemic stroke of unknown cause according to the TOAST criteria [20] were included as controls. They were 43.6 ± 9.3 years old. All CAD and control patients underwent ECG, CT scan, biological examinations including total, HDL and LDL cholesterol levels and homocysteine level, carotid and vertebral artery Doppler sonography, transthoracic and transesophageal echocardiography (performed on control subjects only), cerebral magnetic resonance imaging with T1 fat saturation of the neck, magnetic resonance angiography and cerebral angiography during the acute phase of ischemic stroke (in all patients and controls). All patients and controls received aspirin 300 mg/day.

The endothelium reactivity test has been made at least 80 days after the ischemic stroke to avoid the potential effects of the stoke itself on vascular function.

Exclusion Criteria

Vascular risk factors were collected for both patients and controls. A history of smoking during the previous 4 years, arterial hypertension (blood pressure ≥140/80 mm Hg or current treatment), diabetes (glycemia >1.26 g/l or current treatment) and hypercholesterolemia (total cholesterol >220 mg/dl or current treatment) were considered as vascular risk factors. Patients with any one of these factors were excluded.

Endothelial Reactivity Assessment

In humans, flow-mediated vasodilatation (FMD) can be simply achieved by post-ischemic hyperemia induced in the brachial artery [16]. This arterial vasoreactivity test was applied to patients and controls after a 30-min rest in the supine position in a quiet room at a temperature of 21–23°C. Sudden auditory and visual stimuli were avoided. The brachial artery was imaged with a high-resolution ultrasound imaging system (Acuson 128 XP or Sequoia, Acuson, Mountain View, Calif.) using a high-frequency linear array probe (10–12 MHz). The test was performed in the nonparetic arm. The brachial artery was scanned in the longitudinal section 2–10 cm above the elbow. When the image of both the near and far walls of the vessel was clearly seen and corresponded to the widest lumen diameter, the probe position was drawn on the skin to ensure a fast and accurate repositioning in case of movement. Gray-scale ultrasound sequences were recorded on VHS videotape for off-line assessment. Diameter measurements were achieved at end-diastole coinciding with the R wave of the electrocardiogram.

Distal ischemia was obtained by inflating a pneumatic cuff placed around the forearm, to about 40 mm Hg above the systolic pressure, for 3 min [16]. The artery was scanned for 1 min before inflation to measure the artery diameter at rest (d_0), and 2 min after deflation, to measure the maximal diameter during hyperemia (d_h). Endothelium-dependent vasodilatation was assessed by calculating the flow-mediated index (FMDi) defined as the percentage change in arterial diameters between hyperemia and rest conditions: $FMDi = 100 \times (d_h - d_0)/d_0$.

Endothelium-Independent Vasodilation Assessment

The physiological ability of relaxation of the endothelial vasculature can be assessed by administration of a 'NO donor' like glycerol trinitrine (GTN) which elicits a direct relaxation effect on the white mascular cells of the vessels. We evaluated the endothelium-independent vasodilation by a single dose (400 μg) of sublingual GTN (Leni-

tral Spray, Bessins-Iscovesco, France). GTN was administered after the brachial artery diameter had returned to its initial value. Brachial artery diameter was measured again 5 min after spray administration (d_{gtn}). Endothelium-independent vasodilation was assessed by: $GTNDi = 100 \times (d_{gtn} - d_0)/d_0$.

Statistical Analysis

Data were analyzed using SPSS for Windows V9.0 (Chicago, Ill.). First analyses on demographic data (age) and period between dissection and examination (PDE) were done after assessment of the normality of distribution using a Kolmogoroff-Smirnov test. Results did not allow rejection of the null hypothesis of a normal distribution of age ($p = 0.75$) or of PDE ($p = 0.17$). The Kruskall-Wallis test has been applied to assess the spread homogeneity of age, gender and PDE across the 3 groups, and the resulting χ^2 showed that groups were balanced with subjects' age and gender ($p = 0.19$ and $p = 0.22$, respectively) but not with PDE ($p = 0.043$; table 1). A second analysis was an ANOVA with FMDi and GTNDi as dependent variables and 2 factors (patient group, PDE). Equality of variance was checked using the Levene test. While the Kolmogoroff-Smirnov test did not lead to rejection of the normality of data distribution ($p = 0.16$ for FMDi and $p = 0.44$ for GNDi), the Levene test did not globally allow acceptance of the null hypothesis of data variance homogeneity ($p = 0.05$ for FMDi and $p = 0.72$ for GNDi). As a consequence, nonparametric analysis of variance was used by applying a rank transform to the data. Two non-parametric ANOVAs have then been used: one on the rank of FMDi and the second on the rank of GTNDi, both using 2 factors (patient group and PDE). Post-hoc comparisons were made using the Bonferroni correction for multiple comparisons. The threshold of statistical significance was set at 0.05.

Results

The brachial artery diameter at rest, after hyperemia, and after GTN administration did not differ between the 3 groups. FMDi was 13.8 ± 6.5% in controls, 5.7 ± 6.2% in ICAD and 5.7 ± 8.9% in VAD (table 2). The ANOVA on the ranks of FMDi with 2 factors, group of subjects with 3 levels (ICAD, VAD and controls) and delay with 2 levels (before and after 783 days, median value), showed a significant effect of the group of subjects ($F(82,2) = 15.3$; $p < 0.001$), no effect of delay ($F(82,1) = 2.78$; $p = 0.78$) and no interaction. Post-hoc analysis, using the Bonferroni correction for multiple comparisons, showed a significantly higher FMDi in controls than in ICAD ($p = 0.002$) and VAD ($p < 0.001$), but no significant difference in FMDi between ICAD and VAD patients ($p = 0.28$). The ANOVA and GTNDi with the same factors did not show any significant effect of the group ($p = 0.49$). There were no differences between patients and controls for blood pressure, homocysteine and total cholesterol levels and also for ex-smokers between groups.

Table 1. Number, sex ratio, age (mean ± SD) and period between ischemic stroke and vasoreactivity test (PDE, mean ± SD) in patients with carotid artery dissection (ICAD), vertebral artery dissection (VAD) and controls

	ICAD	VAD	Controls	p
Number (male/female)	26 (17/9)	39 (17/22)	23 (11/12)	0.216
Age, years	41.8 ± 8.06	45.6 ± 7.73	43.6 ± 9.26	0.186
PDE, days	664 ± 556	866 ± 658	1,087 ± 538	0.043

p values were obtained with the Kruskall-Wallis test.

Table 2. Means (± SD) of brachial artery diameters at rest (d_0), during hyperemia (d_h) and after GTN administration (d_{GTN}) for patients with cerebral artery dissection in the carotid (ICAD) and vertebral (VAD) arteries, and in control subjects

	ICAD	VAD	Controls	p
d_0, mm	3.95 ± 0.84	3.82 ± 0.95	3.99 ± 0.90	0.73
d_h, mm	4.15 ± 0.75	3.96 ± 0.87	4.52 ± 1.03	0.11
d_{GTN}, mm	4.76 ± 0.83	4.69 ± 0.90	4.79 ± 0.96	0.89
GTNDi, %	21.5 ± 9.5	25.1 ± 12.5	20.8 ± 8.4	0.49
FMDi, %	5.7 ± 6.2*	5.0 ± 9.3*	13.2 ± 6.5	<0.0005

GTNDi = Percentage of dilation after GTN administration relatively to the diameter at rest; FMDi = flow-mediated dilation index.

* Significant difference vs. controls.

Discussion

This study is the first to describe a significant impairment in endothelium-dependent vasodilation in patients with spontaneous CAD. This impairment is reflected by a decrease in the physiological response to an increase in blood flow. This occurs while vascular dilatory capabilities are preserved as demonstrated by a normal endothelium-independent vasodilation. The impairment of endothelium-dependent vasodilation is not just an acute phenomenon due to stroke as patients were tested at least 80 days after stroke.

We assessed the arterial vasoreactivity with a method widely used to study the effect of vascular risk factors on arterial dilation ability and endothelial function [21]. Flow-mediated vasodilation is now considered a sensitive and specific tool to evaluate endothelial function [22] and used to detect any arterial disease affecting the endothelium. As usually observed in FMD tests [23], in our study

the maximal value of hyperemia occurred within 5 s after a 3-min ischemia or within 5 min after 400-μg GTN administration. FMD is usually assessed relative to the arterial diameter at rest, thus a stable state is mandatory before the onset of the test to get a valuable baseline. Despite their critical impact on the vasomotor state, the stable state conditions are not often controlled or described in the literature. We took special care to warrant a stable state in the same comfortable environmental conditions for all subjects with a long-lasting rest period before measurement.

A significant difference in the FMD index between CAD patients and controls was observed. The endothelium-dependent vasodilatation can thus be regarded as impaired in CAD patients, this happens regardless of the location of the dissection, although ICAD and VAD patients are clinically considered as 2 different subtypes. To our knowledge, no previous study has evaluated endothelial dysfunction in CAD patients. The range of values obtained in CAD patients is similar to that reported in patients with endothelial dysfunction, whatever the etiology.

To avoid a possible effect of stroke on endothelial function, patients were examined after an important delay from the acute clinical event. However, we wanted to address the issue of a possible effect of the delay from the ischemic event itself on endothelial function. A correlation analysis of the FMD index versus delay between stroke and test was performed. There was no significant link between the 3 groups, the slopes of the regression lines being very low as well as the calculated r^2 (ICAD r^2 = 0.02; VAD r^2 = 0.02; controls r^2 = 0.04; $p > 0.05$ in all cases). Actually, the FMD index was not significantly correlated with the delay between ischemic stroke and the test as endothelial dysfunction was present even in case of early (88 days) or very late examination after ischemic stroke (2,383 days). This result supports the hypothesis that endothelial dysfunction is not an acute phenomenon in CAD since it remains over a long period of time, and is no longer a nonspecific consequence of stroke since patients with non-CAD ischemic attack exhibited a normal FMD index. The range of values of the FMDi obtained in this control group is actually similar to that reported in healthy subjects [22–25].

The vascular endothelium normally releases NO, a powerful relaxing factor, in response to many chemical and mechanical stimuli [11, 12, 14]. One important stimulus of the endothelial cell for NO release is the shear stress induced by the mechanical effects of blood flow [15]. The endothelium cell is sensitive to many aggressive vascular factors and its response to shear stress has been shown to be impaired in vascular diseases from their early stages. An impairment in FMD has actually been demonstrated in patients with hypercholesterolemia [19], hypertension [18], diabetes mellitus [26] and smoking [16, 17]. In spontaneous CAD, the preexistence of an underlying tissue disorder has often been evoked. Connective tissue disorders like Marfan's syndrome [27] and polycystic kidney disease [28] have also been associated with CAD. Although these syndromes are rare, a minor form of extracellular matrix defect might be present in CAD patients. Recently, Wilson et al. [29] reported selective impairment of endothelium-dependent vasodilation in subjects with Marfan's syndrome. They proposed a model explaining the relationship between endothelial dysfunction and cytoskeletal abnormalities of the arterial wall, and suggested a modified mechano-transduction of shear stress to NO release. This model might be applied to CAD patients and might explain the observed decrease in FMD in this study.

Considering the hypothesis of a structural origin of CAD, a high risk of recurrence could be expected. However, long-term follow-up studies on CAD patients showed that recurrence is uncommon [30]. Only benign symptoms, such as isolated headache, neck pain, or transient ischemic attack, have been reported in the follow-up. An underestimation of true CAD recurrence is thus possible as these symptoms are not striking and may be neglected by patients and general practitioners. If the low value of spontaneous CAD recurrence is an actual fact, it may be explained by the multifactorial origin of spontaneous CAD, involving a mandatory association of ultrastructural abnormalities with triggering factors like cervical trauma or infection [31].

The major finding of this study is that endothelium-dependent vasodilation is significantly impaired in subjects with spontaneous CAD, whereas endothelium-independent vasodilation is preserved. This finding strongly argues in favor of underlying ultrastructural abnormalities of the intima–media layers, predisposing to spontaneous CAD in some individuals. Future studies on the cellular mechanisms of mechano-transduction could confirm this hypothesis.

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References

1 Cronqvist SE, Norrving B, Nilsson B: Young stroke patients. An angiographic study. Acta Radiol Suppl 1986;369:34–37.
2 Bogousslavsky J, Regli F: Ischemic stroke in adults younger than 30 years of age. Cause and prognosis. Arch Neurol 1987;44:479–482.
3 Bogousslavsky J, Pierre P: Ischemic stroke in patients under age 45. Neurol Clin 1992;10: 113–124.
4 Fisher CM, Ojemann RG, Roberson GH: Spontaneous dissection of cervico-cerebral arteries. Can J Neurol Sci 1978;5:9–19.
5 Hart RG, Easton JD: Dissections of cervical and cerebral arteries. Neurol Clin 1983;1:155–182.
6 Leys D, Lesoin F, Pruvo JP, Gozet G, Jomin M, Petit H: Bilateral spontaneous dissection of extracranial vertebral arteries. J Neurol 1987; 234:237–240.
7 Josien E: Extracranial vertebral artery dissection: nine cases. J Neurol 1992;239:327–330.
8 Amarenco P, Seux-Levieil ML, Cohen A, Levy C, Touboul PJ, Bousser MG: Carotid artery dissection with renal infarcts. Two cases. Stroke 1994;25:2488–2491.
9 Tzourio C, Cohen A, Lamisse N, Biousse V, Bousser MG: Aortic root dilatation in patients with spontaneous cervical artery dissection. Circulation 1997;95:2351–2353.
10 Guillon B, Tzourio C, Biousse V, Adrai V, Bousser MG, Touboul PJ: Arterial wall properties in carotid artery dissection: an ultrasound study. Neurology 2000;55:663–666.
11 Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373–376.
12 Vanhoutte PM, Rubanyi GM, Miller VM, Houston DS: Modulation of vascular smooth muscle contraction by the endothelium. Annu Rev Physiol 1986;48:307–320.
13 Rubanyi GM, Romero JC, Vanhoutte PM: Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986;250: H1145–H1149.
14 Palmer RM, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524–526.
15 Pohl U, Herlan K, Huang A, Bassenge E: EDRF-mediated shear-induced dilation opposes myogenic vasoconstriction in small rabbit arteries. Am J Physiol 1991;261:H2016–H2023.
16 Celermajer DS, Sorensen KE, Gooch VM, et al: Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111–1115.
17 Celermajer DS, Adams MR, Clarkson P, et al: Passive smoking and impaired endothelium-dependent arterial dilatation in healthy young adults. N Engl J Med 1996;334:150–154.
18 Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 1990;323:22–27.
19 Chowienczyk PJ, Watts GF, Cockcroft JR, Ritter JM: Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolaemia. Lancet 1992;340:1430–1432.
20 Adams HP Jr, Bendixen BH, Kappelle LJ, et al: Classification of subtypes of acute ischemic stroke. Definitions for use in a multicenter clinical trial. Stroke 1993;24:35–41.
21 Celermajer DS, Sorensen KE, Georgakopoulos D, et al: Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation 1993;88: 2149–2155.
22 Sorensen KE, Celermajer DS, Spiegelhalter DJ, et al: Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility. Br Heart J 1995;74: 247–253.
23 Leeson P, Thorne S, Donald A, Mullen M, Clarkson P, Deanfield J: Non-invasive measurement of endothelial function: Effect on brachial artery dilatation of graded endothelial dependent and independent stimuli. Heart 1997;78:22–27.
24 Adams MR, Robinson J, Sorensen KE, Deanfield JE, Celermajer DS: Normal range for brachial artery flow-mediated dilatation: A non-invasive ultrasound test of arterial endothelial function. J Vasc Invest 1996;2:146–150.
25 Chao CL, Kuo TL, Lee YT: Effects of methionine-induced hyperhomocysteinemia on endothelium-dependent vasodilation and oxidative status in healthy adults. Circulation 2000;101: 485–490.
26 Goodfellow J, Ramsey MW, Luddington LA, et al: Endothelium and inelastic arteries: An early marker of vascular dysfunction in non-insulin dependent diabetes. BMJ 1996;312:744–745.
27 Hart RG, Easton JD: Dissections of cervical and cerebral arteries. Neurol Clin 1983;1:155–182.
28 Leier CV, Baker PB, Kilman JW, Wooley CF: Cardiovascular abnormalities associated with adult polycystic kidney disease. Ann Intern Med 1984;100:683–688.
29 Wilson DG, Bellamy MF, Ramsey MW, et al: Endothelial function in Marfan syndrome: Selective impairment in flow-mediated vasodilation. Circulation 1999;99:909–915.
30 Leys D, Moulin T, Stojkovic T, Begey S, Chavrot D, for the Donald investigators: Follow-up of patients with history of cervical-artery dissection. Cerebrovasc Dis 1995;5:43–49.
31 Grau AJ, Brandt T, Buggle F, Orberk E, Mytilineos J, Werle E, Conradt, Krause M, Winter R, Hacke W: Association of cervical artery dissection with recent infection. Arch Neurol 1999; 56:851–856.