Increased Carotid Wall Stress in Vascular Ehlers-Danlos Syndrome

Pierre Boutouyrie, MD, PhD; Dominique P. Germain, MD, PhD; Jean-Noël Fiessinger, MD; Brigitte Laloux, PhD; Jérôme Perdu, MD; Stéphane Laurent, MD, PhD

Background—Vascular Ehlers-Danlos syndrome (vEDS), also known as EDS type IV, is an inherited disorder of connective tissue, results from mutations in the gene encoding type III procollagen (COL3A1). Affected patients are at risk for arterial dissection or rupture, the main cause of death. To understand the pathogenesis of the vascular lesions, we used a biomechanical approach and determined steady and pulsatile wall stress.

Methods and Results—Sixteen patients with vEDS and 16 age-, gender-, and blood pressure–matched control subjects were included in this cross-sectional noninvasive study. Circumferential wall stress was determined under steady and pulsatile conditions at the site of an elastic (common carotid) and a muscular (radial) artery from the measurements of intima–media thickness and internal diameter with high-resolution echo-tracking systems and either mean blood pressure or pulse pressure, respectively. At the site of the carotid artery, steady circumferential wall stress was 43% higher in vEDS patients than in control subjects (68.9 ± 14.3 versus 48.2 ± 12.1 kPa, P < 0.001), and pulsatile circumferential wall stress was 22% higher (28.2 ± 7.7 versus 23.1 ± 5.7 kPa, P < 0.001). Carotid intima–media thickness was 32% lower (408 ± 56 versus 598 ± 171 μm, P < 0.001) in vEDS patients, and internal diameter was not different between groups. Radial artery parameters were not significantly different between groups.

Conclusions—In vEDS patients, an abnormally low intima–media thickness generates a higher wall stress than in control subjects at the site of an elastic artery, which may increase the risk of arterial dissection and rupture. (Circulation. 2004; 109:1530-1535.)

Key Words: arteries • carotid arteries • collagen • elasticity • ultrasonics

Ehlers-Danlos syndrome (EDS) type IV (OMIM 130050), the vascular type (vEDS), results from mutations in the gene for type III procollagen.1,2 It is a rare connective tissue disorder inherited as an autosomal dominant trait characterized by 4 main clinical findings: a striking facial appearance, easy bruising, translucent skin with visible veins, and spontaneous rupture of arteries, gravid uterus, or intestines.1–4 Diagnosis is often made only after a catastrophic complication or at postmortem examination. Most deaths result from arterial rupture. The median survival of a recently reported cohort was 48 years.2

Despite identification of the genetic defect, little is known about the pathogenesis of vascular lesions in EDS type IV and the means for preventing arterial complications in young adults. Very few data are available concerning the geometric and elastic properties of conducting arteries in patients with vEDS.5,6 Type III collagen is a member of the fibrillar collagen family and is colocalized with the most abundant member of the family, type I collagen, in such tissues as blood vessels and skin. Mice with mutations in type I and type III collagen were found to die prematurely of ruptured large blood vessels.7,8

In the present study, we used a high-resolution echo-tracking system to noninvasively characterize the arterial phenotype of conducting arteries in patients with vEDS and followed a biomechanical approach to detect changes in arterial wall mechanics favoring rupture.9,10 The vascular phenotype was determined at 2 arterial sites, a proximal predominantly elastic artery, the common carotid artery, and a distal muscular medium-sized artery, the radial artery. Because age, gender, and blood pressure (BP) are 3 major determinants of arterial geometric and elastic properties, we compared vEDS patients with age-, gender-, and BP-matched normal control subjects.

Methods

We included 16 patients (13 women, 3 men) affected with vEDS. The diagnosis of vEDS was made by senior investigators trained and certified in clinical genetics and vascular medicine (D.P.G., J.P., J.-N.F.) on the basis of 4 clinical criteria—facial dysmorphism, excessive bruising/hematomas, thin translucent skin, and rupture of arteries, uterus, or intestines—according to criteria defined in the recently revised nosology for EDS syndromes.1 Facial dysmorphism included acrogeria, prominent bones and sunken cheeks, thin or pinched nose, thin lips, and bulging or protruding eyes often with

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From the Departments of Pharmacology and INSERM EMI 0107 (P.B., B.L., S.L.), Clinical Genetics (D.P.G.), and Vascular Medicine (J.-N.F., J.P.), Hôpital Européen Georges Pompidou, Paris, France.

Correspondence to Pr Stéphane Laurent, Service de Pharmacologie et INSERM EMI 107, Hôpital Européen Georges Pompidou, Assistance Publique–Hôpitaux de Paris, 20, Rue Leblanc, 75015 Paris, France. E-mail stéphane.laurent@egp.ap-hop-paris.fr

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periorbital pigmentation and fine telangiectasis on the eyelids. Data on vascular and visceral complications are given in Table 1.

In 2 patients, the diagnosis was confirmed by the identification of a mutation in the gene coding for type III procollagen (COL3A1). We found the previously reported G514V mutation and another missense mutation (G55D) also involving a glycine residue. Six patients had previous treatment for dyslipidemia (even borderline high) had been made before patients entered the study. No vEDS patient or control subject had diabetes. All patients and subjects were studied in a quiet room with a controlled temperature of 22°C.

### Arterial Parameters

All patients and subjects were matched for age, gender, and BP. No vEDS patient or control subject had previous treatment for dyslipidemia, and no diagnosis of diabetes or hypercholesterolemia was indicated by previous examination, Age at First Episode, and Family History of the Disease. Acrogeria, Excessive bruising, and Translucent Skin are indicated by previous diagnosis of previous treatment for diabetes or hypercholesterolemia.

### Table 1. Clinical Characteristics of Vascular EDS Patients, Including Complications

<table>
<thead>
<tr>
<th>Patients</th>
<th>Gender</th>
<th>Age at Echo Examination, y</th>
<th>Age at First Complication, y</th>
<th>Arterial Dissection or Rupture</th>
<th>Organ Rupture</th>
<th>Family History of the Disease</th>
<th>Acrogeria</th>
<th>Excessive Bruising/ Hematoma</th>
<th>Thin Translucent Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.G.</td>
<td>F</td>
<td>36</td>
<td>28</td>
<td>Carotid-cavernous fistula, iliac artery dissection</td>
<td>Uterus</td>
<td>Yes (son)</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>I.A.</td>
<td>F</td>
<td>31</td>
<td>30</td>
<td>AV fistula tibial artery, stroke</td>
<td>0</td>
<td>Yes (numerous)</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>A.G.</td>
<td>M</td>
<td>26</td>
<td>24</td>
<td>Left verteobasilar dissection</td>
<td>Colonic perforation, coelotomy</td>
<td>No</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>J.S.</td>
<td>M</td>
<td>29</td>
<td>13</td>
<td>0</td>
<td>Total colectomy</td>
<td>Yes (mother)</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>V.C.</td>
<td>F</td>
<td>22</td>
<td>19</td>
<td>0</td>
<td>2 Colonic perforations</td>
<td>No</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>V. P.</td>
<td>F</td>
<td>31</td>
<td>28</td>
<td>0</td>
<td>Uterus</td>
<td>No</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>L.B.</td>
<td>M</td>
<td>14</td>
<td>13</td>
<td>Voluminous hematoma</td>
<td>0</td>
<td>No</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>E.Z.</td>
<td>F</td>
<td>19</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td>No</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R.S.</td>
<td>F</td>
<td>51</td>
<td>50</td>
<td>Splenic artery dissection</td>
<td>0</td>
<td>No</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>J.C.</td>
<td>F</td>
<td>31</td>
<td>N/A</td>
<td>Varicose</td>
<td>0</td>
<td>Yes (father, aunt)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>P.C.</td>
<td>F</td>
<td>46</td>
<td>N/A</td>
<td>Iliac artery dissection</td>
<td>0</td>
<td>Yes (brother)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A.M.</td>
<td>F</td>
<td>17</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td>Yes (mother)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A.M.</td>
<td>F</td>
<td>19</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td>Yes (mother)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>V.L.</td>
<td>F</td>
<td>26</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td>Yes (mother, sister)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C.L.</td>
<td>F</td>
<td>32</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td>Yes (mother, sister)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M.D.</td>
<td>F</td>
<td>45</td>
<td>37</td>
<td>Mesenteric artery aneurysm, renal artery dissection</td>
<td>Colectomy</td>
<td>Yes (mother, uncle)</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

0 indicates absence; +, mild; ++, moderate; and ++++, severe.
Arterial Elastic Properties

The elastic properties of the artery as a hollow structure were assessed through arterial distensibility determined from the systolic-diastolic variations in arterial cross-sectional area (ΔA) and local pulse pressure (ΔP), as previously described, assuming the lumen to be circular. Cross-sectional distensibility coefficient was calculated as DC=ΔA/A·ΔP, where A is the diastolic lumen area, ΔA is the stroke change in lumen area, and ΔP is local pulse pressure. Local carotid and radial artery pulse pressures, directly measured with applanation tonometry, were used in these calculations. The elastic properties of the arterial wall material were estimated by the incremental Young’s elastic modulus (E_{inc}), calculated, as previously described, as E_{inc}=3(1+1/WCSA)/DC, where A is the diastolic lumen area and DC is the cross-sectional distensibility.

Pulse-wave velocity, a classic index of arterial stiffness, was measured along the descending thoracoabdominal aorta with the foot-to-foot velocity method as previously published and validated. Briefly, waveforms were obtained transcutaneously over the common carotid artery and right femoral artery, and the time delay (t) was measured between the feet of the 2 waveforms. The distance (D) covered by the waves was assimilated to the distance measured between the 2 recording sites. Pulse-wave velocity was calculated as PWV = D (meters)/t (seconds).

Statistical Analysis
Quantitative variables were compared by means of a Wilcoxon test. Adjustment of arterial parameters to body surface area (BSA) was based on the general linear model and residual analysis. A Spearman test was used to determine the correlation between carotid and radial IMT. All tests were performed with NCSS 2000 software (J. Hintze, Nashville, Tenn). Data are expressed as mean±SD (minimum–maximum). P<0.05 was considered significant, and tests were 2 sided.

Results
Age, sex ratio, and BP did not significantly differ between vEDS patients and control subjects. Patients with vEDS were shorter and slimmer than control subjects, and BSA was significantly lower in vEDS patients than control subjects (Table 2). Pulse-wave velocity was not significantly different between vEDS patients and control subjects.

Table 3. Carotid Artery Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>vEDS (n=16)</th>
<th>Control Subjects (n=16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal diastolic diameter, mm</td>
<td>5.25±0.45 (4.70–6.11)</td>
<td>5.09±0.48 (4.41–6.22)</td>
<td>NS</td>
</tr>
<tr>
<td>Stroke change in diameter, mm×10⁻³</td>
<td>578±205 (177–929)</td>
<td>543±194 (230–726)</td>
<td>NS</td>
</tr>
<tr>
<td>IMT, mm×10⁻³</td>
<td>408±56 (257–513)</td>
<td>598±171 (417–968)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WCSA, mm²</td>
<td>7.3±1.2 (4.0–9.0)</td>
<td>10.7±3.6 (7.0–19.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Wall-to-lumen ratio</td>
<td>0.16±0.03 (0.11–0.20)</td>
<td>0.24±0.07 (0.13–0.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DC, kPa·⁻¹×10⁻³</td>
<td>61±30 (16–122)</td>
<td>48±20 (15–92)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Young’s elastic modulus, kPa·¹⁻¹</td>
<td>0.27±0.22 (0.08–1.02)</td>
<td>0.23±0.11 (0.08–0.43)</td>
<td>NS</td>
</tr>
<tr>
<td>Steady standard circumferential wall stress, kPa</td>
<td>68.9±14.3 (53.6–110.7)</td>
<td>48.2±12.1 (29.5–78.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Steady midwall circumferential wall stress, kPa</td>
<td>74.4±14.5 (58.9–116.8)</td>
<td>53.5±12.2 (33.8–83.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulsatile wall stress, kPa</td>
<td>28.2±7.7 (13.6–45.2)</td>
<td>23.1±5.7 (14.1–33.0)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

DC indicates cross-sectional distensibility. Values are mean±SD (minimum–maximum).
Carotid IMT was significantly lower (−32%) in vEDS patients than control subjects (Table 3), whereas no significant difference was observed for carotid DI. Accordingly, carotid WCSA and wall-to-lumen ratio were significantly lower in vEDS than in control subjects (Table 3). Steady carotid circumferential wall stress was higher in vEDS than in control subjects (Table 3) when calculated as “standard” wall stress (43% higher in vEDS; Figure 1) or as midwall stress (39% in vEDS). Pulsatile wall stress was 22% higher in vEDS patients than in control subjects. Distensibility was significantly higher in vEDS than in control subjects. Young’s elastic modulus was not significantly different between groups. Except for distensibility and pulsatile wall stress, these differences remained significant after adjustment for BSA.

No significant difference in radial artery parameters was observed between groups (Table 4). Carotid IMT was significantly (P<0.001) related to radial IMT in control subjects but not in vEDS patients (Figure 2).

**Discussion**

The major finding of the present study is the increased carotid circumferential wall stress in patients with vEDS compared with control subjects.

**Interpretation of Findings**

To the best of our knowledge, the present study is the first to characterize the geometric and elastic properties of conducting arteries in patients with vEDS. We used a biomechanical approach to detect changes in arterial wall mechanics favoring dissection and rupture. In vEDS patients, carotid steady circumferential wall stresses, calculated from mean BP, IMT, and either internal diameter or midwall diameter, were 43% and 39% higher, respectively, than in control subjects. In addition, because the fatiguing effect of cyclic stress on the load-bearing elements of the arterial wall, like collagen, is dependent on both the number of cycles and the amplitude of wall stress, we also calculated the latter determinant, ie, pulsatile circumferential wall stress. In vEDS patients, carotid pulsatile circumferential wall stress, calculated from the pulsatile changes in local BP, IMT, and internal diameter, was 22% higher than in control subjects.

The higher circumferential wall stress, either steady or pulsatile, is likely a major factor for dissection and rupture of a fragile arterial tissue. The fragility of the arterial wall in mice with mutations in type I and type III collagens was attributed to the dramatic reduction of the collagen type I fibrils in the aortic media and adventitia. Indeed, type III collagen is not only an essential component of fibrils in tissues such as the media of aorta but also an important regulatory element in type I collagen fibrillogenesis, modulating the size and number of type I collagen fibrils.

In vEDS patients, the fragility of the artery is likely exaggerated by the thinness of the arterial wall, a feature that was not reported in mutant animals. The 32% lower carotid IMT is disproportionate to the reduction in collagen fibers, which represents only a small amount of the wall components. This finding suggests a defect in the control of smooth muscle cell growth and migration and production of extracellular matrix by abnormal collagen type I. Thus, the present study suggests at least 2 mechanisms leading to a reduced load-bearing ability of the arterial wall in vEDS: abnormal collagen type I and abnormal wall thickness, corresponding to abnormal collagen type I fibrillogenesis and abnormal vascular smooth muscle cell signaling, respectively.

Very few data are available on the geometric and elastic properties of conducting arteries in patients with vEDS. Sonesson et al showed no significant difference in carotid stiffness between EDS patients and control subjects, but these authors included patients with various subtypes of EDS and only few vEDS. François et al estimated aortic stiffness from the measurement of pulse-wave velocity in a family with ecchymotic EDS (which actually corresponds to vEDS) and showed abnormally low values of aortic stiffness in 5 relatives. In the present study, carotid-femoral pulse-wave velocity was not significantly reduced, but carotid distensibility was 27% higher in vEDS than in control subjects. In addition, Young’s elastic modulus was not significantly different between the 2 groups despite a higher carotid wall stress in vEDS patients. This finding indicates a shift of the “elastic modulus–wall stress” curve toward lower levels of elastic modulus for a given wall stress and thus increased elasticity of the arterial wall material. This feature of the arterial wall in vEDS may be due to the reduction in the caliber and number of collagen I fibers. It may exaggerate the fatiguing effect of pulsatile stress of the arterial wall biomaterials.

**Methodological Features and Study Limitations**

To ascertain the diagnosis of vEDS, we very carefully selected patients from their clinical follow-up and data on medical files. We retained only those with true acrogeric form because clinical diagnosis was much easier in that case.
Despite careful matching, BSA was lower in vEDS patients than in control subjects and may have accounted for the lower carotid IMT and wall stress. However, this was not the case because differences in IMT and wall stress remained significant after adjustment for BSA in multivariate analysis.

Most of the carotid parameters that were calculated (Table 3) involved the measure of wall thickness, which was significantly lower in vEDS patients than in control subjects. Thus, it was not surprising that most were abnormal. These parameters reflect various aspects of arterial geometry (IMT, WCSA) and function (wall stress).

The adventitia, which represents about one fifth to one fourth of the wall thickness, was not included in measurements and might have been a confounder for the calculation of wall stress. In mice with mutation in type III collagen, collagen fibrils were abnormal not only in the media (absent or severely reduced) but also in the adventitia. Thus, we can reasonably hypothesize that the lower IMT in vEDS patients reflects the lower thickness of the whole vascular wall.

Although carotid artery parameters, measured with the Wall Track system, were abnormal in vEDS, no significant difference between vEDS and control subjects was observed for radial artery parameters, measured with the NIUS 02 system. This was not due to the different characteristics of the 2 systems because they have been tested against each other and both demonstrated a very high precision of measurements, 2.2- to 6.7-fold higher than classic bidimensional echo systems.

Particularly in vEDS, the reduction in IMT was not observed at the site of the radial artery. In control subjects, carotid IMT was positively and significantly correlated with radial IMT (Figure 2), whereas most vEDS patients had carotid IMT values close to 0.4 mm despite a normal range of radial IMT values. This discrepancy was not due to the lack of sensitivity of the NIUS system in measuring low IMT values at the site of a medium-sized artery because several IMT values were measured >120 μm with no correlation with carotid IMT values (Figure 2). Theoretically, an increased difficulty in accurate measurement of IMT ought to be shown in an increased variability. However, the mean SD of 3 to 4 successive IMT measurements was twice lower in vEDS than in control subjects at both sites (P<0.05). The reason may be that the measurements of IMT were more difficult in vEDS than in control subjects; thus, they were even more carefully done than control measurements, and rejection of unacceptable measurements was more frequent.

Thus, another explanation may be the features of the radial artery, which, in contrast to the carotid artery, may not be affected by the abnormal collagen I fibrillogenesis. Because arterial dissections and ruptures also occur at the site of various distal medium-sized muscular arteries, this finding would suggest that an abnormal collagen I fibrillogenesis, by reducing the load-bearing ability of the arterial wall, could lead to the excessive fragility and rupture of the artery despite a normal circumferential wall stress. In addition, we suggest that the likely abnormal vascular smooth muscle cell signaling caused by abnormal collagen type I would be expressed under conditions of high cyclic strain, 18-fold higher at the site of the carotid artery than at the site of the radial artery (Tables 3 and 4). This hypothesis is in line with numerous in vitro studies showing that cyclic strain exerts a greater influence than static load on phenotype and growth of vascular smooth muscle cells, as well as our previous findings that local pulse pressure was a significant determinant of arterial wall thickness at the site of the carotid but not the radial artery.
Clinical Implications of Findings

In patients with vEDS, the diagnosis is often made only after a catastrophic complication or at postmortem examination. Most deaths result from arterial rupture, and the median survival of a recently reported cohort was 48 years.2 The noninvasive arterial phenotype described in the present study, if it had enough sensitivity and specificity, could be useful for evaluating the risk of cardiovascular complications in families with a case, thus leading to appropriate preventive treatment.

Although no preventive treatment has yet been proved to be effective in vEDS, β-blockers are often used by analogy to their efficacy in preventing aortic dissection in patients with Marfan syndrome19 or abdominal aortic aneurysms.20 Although the present study has a cross-sectional design and included a small number of patients, it suggests that patients with high carotid wall stress, either steady or pulsatile, are at high risk for arterial dissection and rupture and that a primary therapeutic goal should be to reduce the amplitude of steady and pulsatile wall stresses, in addition to the classic effects of β-blockers, ie, reducing heart rate and dP/dt (the rate of change in the central BP with respect to time). According to our findings, β-blockers with vasodilating properties should be preferred because they reduce wave reflections and thus pulse pressure to a larger extent than β-blockers devoid of vasodilating effect.13

In conclusion, in patients with acrogeric vEDS, an abnormally low IMT generates a higher wall stress than in control subjects at the site of an elastic artery and thus may increase the risk of arterial dissection and rupture.

References
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