Enhanced Endothelium-Dependent Vasodilation in Fabry Disease

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Background and Purpose—Fabry disease is an X-linked lysosomal storage disease secondary to deficiency of α-galactosidase A with resulting glycolipid accumulation, particularly globotriaosylceramide in arterial smooth muscle and endothelial cells. A systemic vasculopathy, including early-onset stroke, is prevalent without a clear pathogenesis.

Methods—Seventeen normotensive and normocholesterolemic hemizygous Fabry patients (aged 21 to 49 years) and 13 control subjects (aged 21 to 48 years) were investigated by venous plethysmography, allowing assessment of forearm blood flow. Plethysmographic measurements were obtained at baseline and during intra-arterial infusion of acetylcholine and sodium nitroprusside both with and without N\(^{\text{G}}\)-monomethyl-L-arginine (L-NMMA).

Results—Forearm blood flow was significantly higher in patients than in control subjects at all 3 acetylcholine doses (\(P=0.014\)). Patients had a greater response to acetylcholine even after the addition of L-NMMA (\(P=0.036\)).

Conclusions—These results demonstrate an increased endothelium-mediated vascular reactivity in Fabry disease. The increased vessel response to acetylcholine with and without L-NMMA suggests altered functionality of non-NO endothelium-dependent vasodilatory pathways. (Stroke. 2001;32:1559-1562.)

Key Words: acetylcholine ■ blood flow ■ endothelium-derived relaxing factors ■ lipids ■ nitric oxide ■ vasodilation

Fabry disease is an X-linked recessive abnormality of glycosphingolipid metabolism that is due to deficiency of the lysosomal enzyme α-galactosidase A, resulting in the systemic deposition of glycosphingolipids, particularly globotriaosylceramide. Lipid deposition occurs preferentially in vascular endothelium and smooth muscle cells with progressive accumulation leading to ischemia and vessel occlusion. Clinical manifestations seen in hemizygotes with absent, or very reduced, levels of α-galactosidase A activity include pain in the distal extremities (acroparesthesia), which usually begins during adolescence, skin and mucosal angiokeratomas, anhidrosis, corneal opacities, and retrolenticular cataracts. With increasing age, renal failure, strokes, and ischemic heart disease are the leading causes of death around the fourth to fifth decade. The vasculopathy of Fabry disease, resulting in stroke of early onset, is attributed to the progressive deposition of globotriaosylceramide in the vascular endothelium and smooth muscle cells. Although structural compromise to the cerebral, renal, and cardiac arterial vasculature is believed to play a major role in the ischemic events, the pathophysiology of strokes and other events in Fabry disease is unclear. Both hemizygous and heterozygous patients have a higher incidence of both venous and arterial intravascular thrombosis, suggesting that factors involved in thrombosis at the blood-endothelial interface may play a significant part in the occurrence of the vascular events. Increased levels of endothelial prothrombotic factors and leukocyte adhesion-molecule expression have recently been demonstrated in Fabry disease, whereas functional arterial reactivity has not been previously studied.

It is known that endothelial cells modulate arterial vascular tone by releasing contracting and relaxing substances. Furchgott and Zawadzki have demonstrated that endothelium-dependent reactivity of vascular smooth muscle is mediated through endothelium-derived relaxing factor or NO. This regulatory action of the endothelium is abnormal in certain cardiovascular conditions associated with an increased risk of premature development of atherosclerosis. For example, patients with hypercholesterolemia or hypertension are known to have endothelial dysfunction expressed as a blunted vascular response to acetylcholine (ACh) even in the absence of overt vascular disease. We hypothesized that a similar endothelial dysfunction resulting in a decreased response to ACh may contribute to the pathogenesis of ischemic events in Fabry disease.
Subjects and Methods
Seventeen hemizygous Fabry patients (mean age, 31 years) and 13 age- and sex-matched control subjects (mean age, 34 years) were enrolled. The National Institutes of Health Investigational Review Board approved the study, and all participants gave written informed consent. Patient exclusion criteria included a prior history of stroke, cardiac disease, a creatinine clearance of >50 mL/min, and untreated hypertension. Homocysteine levels were not routinely drawn at the time of the study but were available from routine evaluation of the Fabry patients. Homocysteine was assayed in the control group. The α-galactosidase A activity in all patients was <1% of normal. Because both hypertension and hypercholesterolemia are associated with endothelial dysfunction, participants were included in the present study only if the total fasting plasma cholesterol level was <200 mg/dL and the blood pressure was in the normal range (≤140/90 mm Hg). All the subjects in the study were nonsmokers.

Measurement of Forearm Blood Flow by Venous Plethysmography
Each study participant had a total of 4 separate intra-arterial infusions as follows: ACh with and without L-\(^{N}\)-monomethyl-L-arginine (L-NMMA) and sodium nitroprusside (SNP) with and without L-NMMA. Basal measurements were obtained after a 3-minute infusion of 5% dextrose solution at 1 mL/min. Forearm flows were then measured after the infusion of SNP and ACh. SNP was infused at 0.8, 1.6, and 3.2 μg/min, and ACh chloride (Sigma Chemical Co) was infused at 7.5, 15, and 30 μg/min (infusion rates, 0.25, 0.5, and 1 mL/min, respectively, for each drug). Each dose was infused for 5 minutes, and forearm flow was measured during the last 2 minutes of the infusion. A 30-minute rest period was allowed, and another basal measurement was obtained between the infusion of the 2 drugs. After another 30-minute rest period, flow measurements were obtained to corroborate return to basal values. Then, L-NMMA was infused at 4 μmol/min (infusion rate, 1 mL/min) for 5 minutes, and forearm blood flow was measured during the last 2 minutes of the infusion. Subsequently, cumulative dose-response curves for ACh and SNP were repeated with use of the same doses, infusion rates, and resting intervals mentioned above. The infusion of L-NMMA was discontinued during the rest period but reinstated before obtaining the second of these dose-response curves. The sequence of administration of ACh and SNP, both before and after infusion of the arginine analogue (L-NMMA), was randomized to avoid any bias related to the order of drug infusion. During the study, the participants did not know which drug was being infused. All blood pressures were recorded directly from the intra-arterial catheter immediately before each measurement. Testing was performed at an ambient temperature of ~22°C.

Plasma Norepinephrine and Epinephrine Levels
Thirty minutes after placement of the arterial line, each subject had 5 mL of blood drawn into EDTA tubes on ice for immediate delivery to the reference laboratory. Normal reference ranges in the Mayo Clinic, Rochester, Minn, for plasma epinephrine and norepinephrine are 25 to 50 pg/mL and 150 to 350 pg/mL, respectively.

Statistical Analysis
Analysis was performed by ANOVA of repeated measures, allowing statistical comparison between the groups. Drug and dose responses were compared by \( t \) test. A value of \( P<0.05 \) was considered significant.

Results
Patients and control total fasting plasma cholesterol levels were 180±15 and 183±16 mg/dL (normal range 100 to 199 mg/dL), respectively. The mean intra-arterial blood pressure measurements were 76.5±7.1 and 81.6±10.4 mm Hg, respectively. Homocysteine levels were not elevated in the Fabry group.

ACh produced an increase in forearm blood flow in both groups, with a significantly higher increase in the patient group (\( P=0.0149 \), Figure 1A and Table). No significant group difference in the estimated blood flow was found for SNP (Figure 2A and Table). L-NMMA infusion induced a

<table>
<thead>
<tr>
<th>Experimental Mean Blood Flow by Venous Plethysmography</th>
<th>After Intra-Arterial Infusion</th>
</tr>
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<tr>
<td>Mean Blood Flow, mL·min(^{-1})·100 mL Forearm Tissue(^{-1})</td>
<td>Controls</td>
</tr>
<tr>
<td>ACh assessment</td>
<td></td>
</tr>
<tr>
<td>Baseline (before ACh)</td>
<td>2.97±1.14</td>
</tr>
<tr>
<td>ACh1</td>
<td>4.71±2.35</td>
</tr>
<tr>
<td>ACh2</td>
<td>7.20±2.48</td>
</tr>
<tr>
<td>ACh3</td>
<td>10.54±4.31</td>
</tr>
<tr>
<td>L-NMMA (baseline)</td>
<td>-1.51±0.99</td>
</tr>
<tr>
<td>(L-NMMA + ACh1) –baseline</td>
<td>1.10±1.45</td>
</tr>
<tr>
<td>(L-NMMA + ACh2) –baseline</td>
<td>2.56±2.11</td>
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<tr>
<td>(L-NMMA + ACh3) –baseline</td>
<td>7.26±4.26</td>
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<tr>
<td>SNP assessment</td>
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<tr>
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<td>SNP1</td>
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<tr>
<td>SNP3</td>
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<tr>
<td>L-NMMA (baseline)</td>
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<td>(L-NMMA + SNP1) –baseline</td>
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</tr>
<tr>
<td>(L-NMMA + SNP2) –baseline</td>
<td>4.51±1.66</td>
</tr>
<tr>
<td>(L-NMMA + SNP3) –baseline</td>
<td>7.56±2.71</td>
</tr>
</tbody>
</table>

Values are mean±SD. Dose regimen was as follows: ACh1 7.5 μg/min, ACh2 15 μg/min, ACh3 30 μg/min, L-NMMA 4 μmol/min, SNP1 0.8 μg/min, SNP2 1.6 μg/min, and SNP3 3.2 μg/min.

Figure 1. Mean blood flow at baseline and after intra-arterial infusion of ACh (A) and change in mean blood flow after intra-arterial infusion of ACh and L-NMMA (B).
decrease in the resting forearm blood flow in both the patient and the control groups. There was a trend toward a decreased vasoconstrictive response after L-NMMA in patients compared with control subjects \((P=0.06)\).

L-NMMA infusion blunted the vasodilator response to ACh to a similar extent in both groups. Consequently, the response to ACh during NO inhibition was still significantly greater in Fabry patients compared with control subjects \((P=0.0361\text{, Figure 1B and Table})\). L-NMMA did not have any significant effect on the vasodilator response to SNP in either group (Figure 2 and Table).

There was no statistical difference between the groups in the plasma epinephrine levels (Fabry patients, 46.5±31.6 pg/mL; control group, 41.8±28.6 pg/mL) or norepinephrine levels (Fabry patients, 151.1±60.1 pg/mL; control group, 141.7±54.5 pg/mL).

**Discussion**

Fabry patients have an increased forearm blood flow response to intra-arterial ACh compared with control subjects. A similar blunting of the ACh response after L-NMMA infusion was found in both groups. Furthermore, there was no statistical difference in the response to SNP with or without L-NMMA between the patient and control groups.

A prominent feature of Fabry disease is a distal small fiber neuropathy, and it is known that lamellated glycolipid inclusions bodies occur in the small neurons of peripheral sensory and autonomic ganglia.\(^{11}\) Plasma norepinephrine results from spillover secondary to postganglionic adrenergic nerve terminal activity.\(^{12}\) The net functional integrity of the sympathetic nervous system may be estimated from the supine plasma norepinephrine level. We found no difference in the control and patient values of plasma norepinephrine, suggesting an intact sympathetic neuronal innervation of the peripheral vasculature in the Fabry patients studied. Further neurological examination demonstrated, at most, loss of cold and warm sensations in the distribution of the common peroneal nerve. Nerve conduction studies were within normal limits.

Therefore, the altered vessel response found in Fabry disease may be attributed to vasogenic as opposed to neurogenic factors. The effect of ACh was compared with the effect of SNP, a direct activator of vascular smooth muscle guanylate cycle.\(^{6–8}\) The contrasting effect of ACh and SNP allows differentiation of endothelium-dependent and direct smooth muscle vasodilation. The exaggerated response to ACh in Fabry patients demonstrates increased endothelium-dependent vasodilation, whereas the normal response to SNP rules out the possibility that the response to ACh is due to enhanced smooth muscle reactivity to vasodilator stimuli.

Because the endothelium-dependent response to ACh may be mediated not only by NO but also by other endothelial factors, we analyzed the effect of NO synthesis inhibition on forearm blood flow and in response to ACh with the competitive inhibitor of L-arginine, L-NMMA. Infusion of L-NMMA at 4 \(\mu\)mol/min results in inhibition of the endothelial NO pathway; thus, the ACh-induced vasodilation after L-NMMA inhibition is consistent with activity in non-NO pathways.\(^{13–16}\) These pathways are known to be preferentially responsive in resistance vessels, especially when there is impaired NO-dependent endothelial function.\(^{16–18}\) The plethysmographic determination of forearm blood flow is dominated by the resistance vasculature, so that the observed response to ACh in Fabry disease is secondary to altered resistance vessel function. The fact that the inhibitor L-NMMA showed less vasoconstriction in patients compared with control subjects \((P=0.06)\) also suggests that the NO pathway may be downregulated in patients with Fabry disease, allowing dominance of the non-NO pathways.\(^{17,18}\) If the NO pathway had been equifunctional in the Fabry group and the control group, a similar vasoconstrictive response would have been expected. It is possible that the size of our subject groups did not provide sufficient power to demonstrate a significant difference. The similar effect of L-NMMA on the ACh response in both groups together with the resulting greater response to ACh during NO inhibition further suggests an imbalance between the NO and non-NO endothelial pathways in Fabry disease. Because of the very short circulatory activity of ACh and SNP together with the dosage regime used, it would seem unlikely that any part of the observed response is attributable to a systemic effect.

The connection between abnormal endothelium-dependent vascular reactivity and the dolichoectasia typical of Fabry vasculopathy is unclear. It is possible that an excessive vascular response to normal hemodynamic stress could result in vessel wall remodeling with the development of vessel tortuosity and that occlusive disease may be more related to glycosphingolipid in the vessel wall.

In conclusion, these findings suggest that other (non-NO) factors determine the exaggerated response to ACh in Fabry patients and have a greater-than-normal role in the regulation of their vascular tone. This endothelial functional abnormality likely underlies the hyperdynamic cerebral circulation that we observed in Fabry patients by use of positron emission tomography and transcranial Doppler techniques.\(^{19,20}\) It is
important to emphasize that the enhanced endothelium-dependent vasodilation was demonstrated in the forearm vascular bed. We do not at present have any evidence that a similar abnormality exists in the cerebrovascular bed, although positron emission tomography and transcranial Doppler techniques support this view. Among the possible candidates for this non-NO factor is endothelium-derived hyperpolarizing factor. Its nature is currently unknown, but previous studies have suggested that endothelium-derived hyperpolarizing factor may take a greater role in vascular tone modulation in the context of defective NO activity. The finding of a disturbed relationship between the endothelial NO and non-NO pathways may allow a greater understanding of the pathophysiology of the vasculopathy in Fabry disease.

Acknowledgment

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References

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