Impaired Cerebral Vasoreactivity in a Transgenic Mouse Model of Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy Arteriopathy

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Background and Purpose—Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited small vessel disease causing stroke and dementia. The disease is caused by highly stereotyped mutations in NOTCH3, which is restrictively expressed in vascular smooth muscle cells (VSMCs). The mechanisms of compromised cerebral hemodynamics in CADASIL remain to be elucidated. We tested the hypothesis that mutant NOTCH3 impairs the vasomotor function of cerebral vessels.

Methods—Vasomotor function was examined in vivo in transgenic mice expressing a mutant NOTCH3 in VSMCs (TgNotch3R90C). Mice develop an age-dependent arteriopathy similar to that seen in CADASIL, without brain parenchyma lesions. Using laser-Doppler flowmetry, we assessed in awake TgNotch3R90C mice and wild-type littermates the cerebrovascular reactivity to 2 potent vasodilator stimuli (acetazolamide and hypercapnia) and cerebral blood flow (CBF) autoregulation during stepwise blood pressure elevations and reductions. Mice were studied at 18 months of age, when the CADASIL features are apparent, and at 10 months of age, before their appearance.

Results—Eighteen-month-old TgNotch3R90C mice showed reduced responses to hypercapnia and acetazolamide, higher cerebrovascular resistance during hypertension, and their lower limit of CBF autoregulation was shifted to higher blood pressures. Cerebrovascular responses were similarly impaired in 10-month-old TgNotch3R90C mice.

Conclusions—Cerebrovascular reactivity is compromised early in TgNotch3R90C mice. The data show an impaired autoregulation and are suggestive of a decreased relaxation or increased resistance of cerebral vessels. Our findings indicate that vascular dysfunction is an early pathogenic event that may promote the subsequent development of brain ischemia in CADASIL. (Stroke. 2005;36:1053-1058.)

Key Words: autoregulation ■ CADASIL ■ laser-Doppler flowmetry

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited small vessel disease caused by highly stereotyped mutations in the NOTCH3 gene. Clinical manifestations include recurrent ischemic strokes and cognitive impairment leading to subcortical dementia and premature death. MRI of the brain displays T2-weighted hyperintensities within the periventricular white matter in asymptomatic carriers. Later, T1-weighted images show multiple lacunar infarcts in the white matter and basal ganglia. The underlying vasculopathy is characterized by degeneration of vascular smooth muscle cells (VSMCs), characteristic deposition of granular osmiophilic material (GOM) within their basement membrane, and accumulation of Notch3 protein at their cell membrane. VSMCs have been shown to be the primary targets of the pathogenic process of CADASIL.

Positron emission tomography, MRI bolus tracking, or phase contrast in CADASIL patients revealed that cerebral blood flow (CBF) was reduced, especially in the white matter, and that impaired flow may precede white matter hyperintensities. As yet, the mechanisms of compromised cerebral hemodynamics are unknown. The commonly proposed hypothesis is that flow deficiency results from vascular abnormalities, such as narrowing or occlusion, distortion, or loss of small arteries and capillaries. Alternatively, it has been suggested that VSMC alterations may be responsible for vascular dysfunction.

The purpose of this study was to test the hypothesis that expression of mutant NOTCH3 in VSMCs impairs the vasomotor function of cerebral vessels. We recently generated transgenic mice expressing an archetypal CADASIL mutant Notch3 (TgNotch3R90C) specifically in VSMCs. Transgenic mice recapitulate the preclinical stage of the CADASIL disease. Specifically, mutant mice develop an age-dependent arteriopathy, similar to that seen in asymptomatic NOTCH3 mutation carriers, but develop neither the brain...
TABLE 1. Resting Physiological Parameters in the 2 Lines of 18-Month-Old TgNotch3R90C and Wild-Type Littermates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ntg Mice (n=9)</th>
<th>TgVe Mice (n=7)</th>
<th>TgMa Mice (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mo</td>
<td>18.2±0.1</td>
<td>18.4±0.2</td>
<td>18.2±0.1</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>34.9±2.5</td>
<td>33.1±2.6</td>
<td>35.0±1.8</td>
</tr>
<tr>
<td>MAPB, mm Hg</td>
<td>95.2±1.4</td>
<td>103.3±2.1*</td>
<td>93.8±2.7</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>580±16</td>
<td>633±16</td>
<td>588±18</td>
</tr>
<tr>
<td>Arterial blood pH</td>
<td>7.33±0.01</td>
<td>7.35±0.01</td>
<td>7.34±0.01</td>
</tr>
<tr>
<td>PaCO2, mm Hg</td>
<td>41.6±2.0</td>
<td>42.8±1.4</td>
<td>40.5±1.2</td>
</tr>
<tr>
<td>PaO2, mm Hg</td>
<td>113.3±3.6</td>
<td>111.1±3.5</td>
<td>111.7±2.3</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>43.8±0.8</td>
<td>42.1±1.3</td>
<td>43.3±1.1</td>
</tr>
</tbody>
</table>

TgVe and TgMa are TgNotch3R90C lines; Ntg are wild-type littermates. Values are means±SE; n indicates the number of mice; MAPB and heart rate values are the average of 2 measurements per mouse; arterial blood was analyzed before intravenous infusions.

*Compared with age-matched nontransgenic mice (ANOVA; P<0.02; Tukey test).

parenchyma lesions nor the clinical symptoms observed in CADASIL patients. Using laser-Doppler flowmetry (LDF), we assessed in TgNotch3R90C and wild-type littermates mice the cerebrovascular reactivity to acetazolamide and hypercapnia, which are potent vasodilator stimuli. We also examined CBF autoregulation, as performed previously in the mouse. Autoregulation reflects a fundamental property of the cerebral circulation that enables it to maintain stable brain perfusion in face of blood pressure changes. Autoregulation depends on the ability of resistance vessels to dilate when mean arterial blood pressure (MAPB) falls and to constrict when MAPB rises. Importantly, all experiments were conducted on awake mice to avoid any interference of anesthetics agents with cerebrovascular reactivity.

Materials and Methods

Animals

The generation and characterization of transgenic mice expressing human Notch3 with the arginine-to-cysteine missense mutation at position 90 (TgNotch3R90C) under the control of the arterial smooth muscle cell-specific SM22α promoter have been described in detail previously. Two independent transgenic founder lines, transgenic mauve and transgenic verte, expressing 20% and 200% of endogenous Notch3 accumulation) were present in the mouse. Autoregulation curves. Because interaction between pressure steps and groups was significant, the differences at each pressure step were evaluated by a 1-way ANOVA and Tukey test. Values were expressed as means±SE.

Statistical Analysis

Mice of 10 and 18 months of age were studied alternately. Physiological parameters and CBF changes at each age were compared by ANOVA (1- or 2-factor, as appropriate; P of ANOVA is given) and Tukey test for multiple comparisons. Repeated-measures ANOVA was used to compare profiles of the responses to acetazolamide and autoregulation curves. Because interaction between pressure steps and groups was significant, the differences at each pressure step were evaluated by a 1-way ANOVA and Tukey test. Values were expressed as means±SE.

Results

Cerebrovascular Reactivity in 18-Month-Old Transgenic Mice

At the age of 18 months, vascular CADASIL features (ie, GOM deposits and Notch3 accumulation) were present in the brain vessels of TgNotch3 mice in the absence of parenchymal lesions.

Physiological Parameters

Under resting conditions, MAPB of TgMa mice was similar to that of wild-type (Ntg) mice. In TgVe male and female mice, MAPB and systolic and diastolic pressures were slightly but significantly higher than Ntg littermates. Other
parameters, including body weight, heart rate, blood gases, and hematocrit, were similar in transgenic and Ntg mice (Table 1).

Cerebrovascular Responses to Acetazolamide and Hypercapnia

Intravenous administration of acetazolamide increased CBF without altering MABP. In Ntg mice, CBF increase reached a maximal value of $56.2\pm 4.0\%$ 5 minutes after injection and lasted up to 30 minutes (Figure 1A). In TgMa and TgVe mice, CBF increase was significantly attenuated (Figure 1A and 1B). In Ntg mice, hypercapnia increased CBF by $4.0\%$ per mm Hg of PaCO$_2$ and decreased CVR by $2.2\%$ per mm Hg of PaCO$_2$. Hypercapnic hyperemia was attenuated by $50\%$ in TgMa and TgVe mice (Figure 1C).

Cerebrovascular Responses to MABP Elevation

Phenylephrine injection ($40 \mu g/kg$) increased MABP to a similar level in Ntg and TgMa mice and to a slightly higher level in TgVe mice, although not significantly so. These acute hypertensions were associated with lesser CBF increases in TgMa and TgVe mice than in Ntg mice (Figure 2A). Consistently, CVR increases were clearly stronger in both transgenic lines (Figure 2B).
VSMCs exhibit an impaired cerebral vasoreactivity, comprising in transgenic mice expressing a CADASIL mutant NOTCH3 in significance. In this study, we provide strong evidence that given partly controversial results of unclear pathogenic significance. Investigation of cerebrovascular function in CADASIL has been an early pathogenic event that may promote the subsequent development of ischemia in brain parenchyma in CADASIL. Therefore, it is likely that cerebrovascular dysfunction arises from VSMC dysfunction rather than from VSMC degeneration. Hence, it is conceivable that attenuation of vasodilator responses may lead to a poorer adaptation to local or global increases in blood flow when metabolic needs require a higher blood supply. Likewise, impaired CBF autoregulation may render the brain more susceptible to hypotension, in accordance with a scenario already suspected to promote ischemic insults in CADASIL.17 Collectively, our findings strongly support the notion that vascular dysfunction is an early pathogenic event that may promote the subsequent development of ischemia in brain parenchyma in CADASIL.

Alterations of cerebral autoregulation similar to those observed in TgNotch3R90C mice have been well documented in hypertensive animals.18 Although mice from 1 transgenic line of this study exhibited a higher MABP compared with age-matched Ntg littermates, several observations indicate that the vascular dysfunction in TgNotch3R90C mice is not a consequence of hypertension. First, the difference in MABP between aged TgVe mice and controls was modest, 10 mm Hg. Second, altered cerebral hemodynamics were detected in TgVe mice at 10 months of age when blood pressure of these mice was identical to that of age-matched Ntg mice. Third, similarly altered responses were detected in TgMa mice, a distinct transgenic line, which showed normal MABP. The mechanisms responsible for a higher blood pressure in aged TgVe mice remain to be clarified. In fact, MABP in control mice exhibited a decrease in 10-month-old transgenic mice before the appearance of the characteristic vascular alterations of CADASIL. Therefore, it is likely that cerebrovascular vasoreactivity was detected in 10-month-old transgenic mice before the onset of any detectable brain parenchyma lesions and thus cannot be attributed to gross neuropathological abnormalities. Impaired cerebrovascular vasoreactivity was detected in 10-month-old transgenic mice before the appearance of the characteristic vascular alterations of CADASIL. Therefore, it is likely that cerebrovascular dysfunction arises from VSMC dysfunction rather than from VSMC degeneration. Hence, it is conceivable that attenuation of vasodilator responses may lead to a poorer adaptation to local or global increases in blood flow when metabolic needs require a higher blood supply. Likewise, impaired CBF autoregulation may render the brain more susceptible to hypotension, in accordance with a scenario already suspected to promote ischemic insults in CADASIL.17 Collectively, our findings strongly support the notion that vascular dysfunction is an early pathogenic event that may promote the subsequent development of ischemia in brain parenchyma in CADASIL.

Physiological parameters did not differ between 10-month-old transgenic and Ntg littermate mice. Importantly, MABP was similar in TgMa and TgVe and Ntg mice (Table I, available online only at http://stroke.ahajournals.org). The vasodilator responses to acetazolamide and hypercapnia were significantly attenuated in TgMa and TgVe mice (Figure 4A and 4B). Phenylephrine injection induced higher CVR increases in TgMa and TgVe mice compared with Ntg mice (Figure 4C). The upper limit of CBF autoregulation (110% of baseline) was similar in Ntg and TgMa mice (124 and 126 mm Hg, respectively) and shifted to higher blood pressures (136 mm Hg) in TgVe mice. The lower limit of CBF autoregulation (90% of baseline) was shifted from 60 mm Hg to higher blood pressures: 75 and 85 mm Hg in TgMa and TgVe mice, respectively (Figure 4D).
Cerebrovascular responses to acute elevations or reductions of blood pressure involve essentially myogenic mechanisms, modulated by neurogenic and metabolic influences. Responses to hypercapnia and acetazolamide involve a decrease in perivascular pH, the factor of which acts on VSMCs with no or only minor contribution of the vascular endothelium. Thus, findings in TgNotch3R90C mice are suggestive of an impairment of predominantly the myogenic response, with decreased relaxation or increased resistance of cerebral vessels. These in vivo data are consistent with our recent data on the reactivity to mechanical forces and pharmacological stimuli of isolated systemic arteries from the same TgNotch3R90C mice. Specifically, we showed that caudal arteries from 10-month-old TgNotch3R90C mice exhibited a significant increase in pressure-induced contraction and a significant decrease in flow-induced dilation. In contrast, phenylephrine-induced constriction and acetylcholine-induced dilation were unaffected in these mice, indicating that the defective transduction of mechanical forces did not arise from a global dysfunction of VSMCs. Pressure and flow are 2 mechanical stimuli that determine the basal vascular tone in resistance arteries. Our data support the idea of an increased vascular tone in transgenic mice, at least in the caudal and cerebral arteries, because of a primary dysfunction of VSMCs expressing a mutant NOTCH3. The specific molecular basis for this defect is as yet unclear. Ultrastructural analyses are consistent with the possibility of an increased actin polymerization in VSMCs of mutant arteries.

In conclusion, the present study provides evidence that expression of a mutant NOTCH3 in VSMCs early compromises cerebrovascular reactivity. Whether such functional deficits result in acute or chronic hypoperfusion and subsequent brain parenchyma damages remains to be investigated.

Acknowledgments

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References


