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

Pierre Lacombe, PhD; Charleen Oligo; Valérie Domenga; Elisabeth Tournier-Lasserve, MD; Anne Joutel, MD, PhD

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
TABLE 1. Resting Physiological Parameters in the 2 Lines of 18-Month-Old TgNotch3R90C and Wild-Type Littermates

<table>
<thead>
<tr>
<th></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>

PaCO2, mm Hg 41.6±0.01 7.35 100
Arterial blood pH 7.33 100
PaO2, mm Hg 113.3±3.6 111.1±3.5 111.7±2.3

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 anesthetic 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 murine NOTCH3 in the brain, respectively, were established on a C57BL/6 background. Male and female mice heterozygous for the transgene and their wild-type littermates (nontransgenic [Ntg]) were used (n=51). Studies were performed in age-matched TgNotch3R90C mice and control littermates at 10 and 18 months of age. All animal procedures followed the European Community standards on the care and use of laboratory animals (Prefecture de Paris; authorizations 75-071 to P.L. and 75-572 to A.J.) and have been approved by the local ethics committee (Ile-de-France-Paris, Comité Régional d’Ethique n°4).

Animal Preparation

Mice were anesthetized with isoflurane (2.0% progressively reduced to 1.7%) in a mixture of O2 and N2 inhaled via a facemask. A femoral artery was catheterized for arterial blood pressure, heart rate, and arterial blood gas measurements. A femoral vein was catheterized for drug infusions. Catheters were heparinized so as to provide 1000 IU/kg. Skin incisions were carefully sutured and covered with 2% lidocaine gel. CBF was monitored by LDF (Moor Instruments; MBF3-Dual). Two LDF probes were stereotaxically placed and sealed above the parietal bone, away from large pial vessels. Mice were held under minimal restraint and allowed to recover from anesthesia. Rectal temperature was kept at 37.4±0.1°C. Heart rate, blood pressure, and CBF were continuously monitored using a multichannel recorder (RS 3400; Gould). Mice were habituated to anesthesia and restraint on the day before experiment.

Experimental Protocol

Experiments were performed on awake animals and started =2 hours after isoflurane was interrupted. Hypercapnia was induced by adding 5% to 7% CO2 to the inhaled O2/N2 gas mixture for 2 minutes. Arterial blood was sampled at the end of the 2 minutes for blood gas analysis (Ciba-Corning 248 blood gas analyzer). Acetazolamide was injected intravenously as a bolus (7 mg/kg). As designed previously in mice, CBF autoregulation was investigated in elevating MAPB by intravenous infusion of phenylephrine (15 to 40 μg/kg) up to 150 mm Hg. Hypertension above this level was not attempted because this may result in pulmonary edema. Hypotension was induced by controlled, stepwise withdrawal of 100 μL of arterial blood down to 30 mm Hg. Blood pressure was allowed to stabilize during 2 to 3 minutes at each pressure step. All mice underwent the same experimental protocol, including first MAPB elevation, second hypercapnia, and third acetazolamide infusion, allowing CBF and MAPB to stabilize for 10 to 15 minutes before conducting the subsequent test. The fourth and last challenge, controlled hypotension, was performed =1 hour after acetazolamide infusion. A total volume of 200 to 220 μL of arterial blood was drawn under mouse for blood gas analyses and hematocrit measurements.

Data Analysis

Flow values from the 2 LDF probes were averaged for each mouse. Changes in CBF were expressed as percent change from baseline. Response to acetazolamide was quantified by determining the area under the curve of CBF percent changes. CO2 reactivity was calculated by dividing the maximum increase in CBF% to the increase in arterial PCO2 (mm Hg) during hypercapnia. Cerebrovascular resistance (CVR) was calculated as the ratio of MAPB to concomitant CBF (CVR=MAPB/LDF), and results were expressed as percent changes of the baseline CVR: Δ%CVR=100 [(CVRbaseline−CVRinfusion)/CVRbaseline]. The upper and lower limits of CBF autoregulation were defined as the MAPB at which CBF goes >110% or <90% of the baseline value, respectively.

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 ± 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 PaCO2 and decreased CVR by 2.2% per mm Hg of PaCO2. Hypercapnic hyperemia was attenuated by 50% in TgMa and TgVe mice (Figure 1C).

Cerebrovascular Responses to MABP Elevation
Phenylephrine injection (40 μg/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).

We further studied the relationship between MABP and CBF in determining the upper limit of CBF autoregulation. In response to stepwise hypertensions, CBF did not exceed 110% of baseline, up to 118 mm Hg in Ntg mice. The upper limit of CBF autoregulation was similar in TgMa mice...
VSMCs exhibit an impaired cerebral vasoreactivity, comprising partly controversial results of unclear pathogenic significance. In this study, we provide strong evidence that given the stepwise hypotensions down to 60 mm Hg and decreased steeply thereafter. In both lines of transgenic mice, the lower limit of CBF autoregulation was significantly shifted to higher MABP: 77 and 87 mm Hg in TgMa and TgVe mice, respectively (Figure 3, left side of the curves).

Cerebrovascular Reactivity in 10-Month-Old Transgenic Mice

To determine whether cerebrovascular reactivity was altered before brain vessels develop the CADASIL features, we conducted an identical study in 10-month-old transgenic and Ntg littermate mice. Physiological parameters did not differ between 10-month-old transgenic and Ntg mice (Table 1, available online only at http://strokeaha.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).

Discussion

Investigation of cerebrovascular function in CADASIL has given partly controversial results of unclear pathogenic significance. In this study, we provide strong evidence that transgenic mice expressing a CADASIL mutant NOTCH3 in VSMCs exhibit an impaired cerebral vasoreactivity, comprising reduced responses to vasodilatory challenges, higher CVR during hypertensions, and a shift of the lower limit of CBF autoregulation toward higher blood pressures. Some features of these findings provide insights into the pathogenic significance of the impairments. Vascular dysfunction occurred before the onset of any detectable brain parenchyma lesions and thus cannot be attributed to gross neuropathological abnormalities. Impaired cerebrovascular vasoactivity 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. 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. Although mice from 1 transgenic line of this study exhibited a higher MABP compared with age-matched Ntg littersmates, 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

![Figure 3. Relationship between CBF and MABP in 10-month-old Ntg and TgNotch3R90C mice. MABP was increased (phenylephrine) and decreased (controlled bleeding) in Ntg mice (n = 9), TgVe mice (n = 7), and TgMa mice (n = 9). During bleeding hypotension, CBF fell <90% of baseline (lower dotted line) by 60 mm Hg in Ntg mice, and by 77 and 87 mm Hg in TgMa and TgVe mice, respectively, indicating impaired autoregulation. In response to phenylephrine, CBF remained <110% of baseline (upper dotted line), up to 120 mm Hg in Ntg and TgMa mice, and up to 135 mm Hg in TgVe mice. The MABP range for which CBF was statistically different from Ntg mice was 24 to 89 mm Hg for TgVe mice, 24 to 65 mm Hg for TgMa mice in the lower part of the curve, and 121 to 137 mm Hg for TgVe mice in the upper part of the curve. Trend curves were obtained with the polynomial regression (5th degree) providing the best fit (all values of P < 0.05). ANOVA, P = 0.001; * and #, TgVe and TgMa vs Ntg mice, respectively; Tukey’s test.](image-url)
suggestive of an impairment of predominantly the 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 contraction 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. Additional studies are required to address this specific issue.

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

This work was supported by grants from the CADASIL Foundation of America, the GIS Maladies Rares, and the Fédération pour la Recherche sur le Cerveau to A.J. The authors thank Dr Eric Vicaut for his help in design of the statistical analysis, and Dr Richard Sercombe for valuable reading of this manuscript.

References


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

Pierre Lacombe, Charleen Oligo, Valérie Domenga, Elisabeth Tournier-Lasserve and Anne Joutel

Stroke. 2005;36:1053-1058; originally published online April 7, 2005;
doi: 10.1161/01.STR.0000163080.82766.eb

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/36/5/1053

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/