Effect of Lovastatin on Cerebral Circulation in Spontaneously Hypertensive Rats

Olivier Régrigny, Jeffrey Atkinson, Christine Capdeville-Atkinson, Patrick Limñana, Jean-Marc Chillon

Abstract—Statins, which are often given to hypertensive patients, reduce the incidence of stroke. However, their effects on the cerebral circulation have been scarcely studied, although lovastatin has been reported to reduce hypertension-induced renal arteriolar hypertrophy. We examined the structure and mechanics of cerebral arterioles and the lower limit of cerebral blood flow (CBF) autoregulation in spontaneously hypertensive rats (SHR) that were untreated (n=9) or treated for 1 month with lovastatin (n=12; 20 mg · kg⁻¹ · d⁻¹) and in untreated Wistar-Kyoto rats (WKY; n=8). We studied the lower limit of CBF autoregulation by repeated measurement of CBF (arbitrary units; laser Doppler) and internal arteriolar diameter (µm; cranial window) at baseline and during stepwise hypotension. Stress-strain relationships were calculated from repeated measurement of internal arteriolar diameter during stepwise hypotension and cross-sectional area (CSA) of the vessel wall in maximally dilated cerebral arterioles (EDTA, 67 mmol/L). Lovastatin slightly reduced mean arterial pressure (treated, 153±3 versus untreated, 171±5 mm Hg, P<0.05; WKY, 106±3 mm Hg) and normalized CSA (treated, 826±52 versus untreated, 1099±16 µm², P<0.05; WKY, 774±28 µm²). Stress-strain curves show that lovastatin also attenuated the increase in passive distensibility. Lovastatin had no effect on the external diameter of cerebral arterioles or the lower limit of CBF autoregulation. Our results show that although lovastatin has substantial effects on arteriolar mechanics and wall CSA, it has little effect on internal diameter. This phenomenon may explain its lack of effect on CBF autoregulation. (Hypertension. 2000;35:1105-1110.)

Key Words: hypertrophy remodeling inhibitors, HMG-CoA reductase autoregulation

Patients with high cholesterol are treated with 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins); at least one third of these patients also suffer from hypertension.¹ Meta-analysis of clinical trials reveals that statins reduce the incidence of ischemic stroke,¹² and the data have been interpreted as evidence of a cholesterol-independent effect of statins on cerebrovascular disease.¹ Lovastatin can reduce renal arteriolar-wall hypertrophy in spontaneously hypertensive rats (SHR).³ However, at the present time, little is known about the effects of statins on cerebral arteriolar structure and mechanics in hypertensive rats and how such effects may modify cerebral blood flow (CBF) autoregulation.

The first goal of the present study was to examine the effects of chronic treatment (1 month) with lovastatin on cerebral arteriolar structure and passive distensibility in SHR. The second goal was to determine whether modification of the structure and passive distensibility has any effect on the lower limit of CBF autoregulation in SHR.

Methods

Animal Preparation

Experiments were performed on male Wistar-Kyoto rats (WKY) and male SHR (Ifa-Credo, l’Arbresle, France). At 10 weeks of age, SHR were divided into 2 groups: a group that received lovastatin in the drinking water (20 mg · kg⁻¹ · d⁻¹; n=12) and a group that received vehicle (n=9). A group of WKY that drank vehicle served as normotensive controls (n=8).

Lovastatin was prepared twice weekly by dissolving the base in 3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, HMG-CoA reductase autoregulation

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A silicone catheter (Sigma Medical) was introduced into a femoral vein and connected to a pump (Bibloblock Scientific) for infusion of sodium pentobarbital (0.25 mL · h⁻¹; 20 mg · kg⁻¹ · h⁻¹) throughout the experiment to maintain anesthesia. Animals were intubated and mechanically ventilated with room air (60 strokes · min⁻¹; 10 mL · kg⁻¹). Paralysis of skeletal muscles was obtained with an injection of gallamine triethiodide (20 mg · kg⁻¹ · IV) repeated every hour. Rectal temperature was maintained at 37°C with a heating pad. Because the animals were paralyzed, the depth of anesthesia was periodically evaluated by application of pressure to the tail and confirmation of the absence of changes to heart rate and blood pressure.

Measurement of Arteriolar Inner Diameter
We measured internal diameter in first-order arterioles of the cerebrum through an open-skull preparation. The head was placed in an adjustable head holder. A 1-cm incision was made in the skin to expose the skull. Ports were placed for inflow and outflow of artificial cerebrospinal fluid (CSF). Craniotomy was performed over the left parietal cortex, and the dura was incised to expose cerebral vessels. The exposed brain was continuously suffused with artificial CSF warmed to 37°C to 38°C and equilibrated with a gas mixture of 5% CO₂:95% N₂. The composition of the CSF was (in mmol/L): KCl 3.0, NaCl 0.6, CaCl₂ 1.5, NaCl 131.9, NaHCO₃ 24.6, urea 6.7, and glucose 3.7. Arterioles were monitored through a microscope (Stemi 200-C; Carl Zeiss Jena GMBH) connected to a closed-circuit video system with a final magnification of ×400. Images of arterioles were digitized using a video-frame grabber. Arteriolar inner diameter was measured from the digitized images by use of image-analysis software (Saisam, Microvision Instruments); the precision of this system is 0.5 μm or 0.4% to 1.2%, depending on measured diameter.

Measurement of CBF
CBF was measured by laser Doppler flowmetry with a BLF 21 system (Transonic Systems Inc.) equipped with a 1.2-mm-diameter needle probe. The probe was placed through the cranial window into the CSF above the surface of the brain stem. CBF was expressed in arbitrary units or as percentage of baseline changes in CBF during stepwise hypotension.

Experimental Protocol
Thirty minutes after completion of surgery, cerebral arteriolar inner diameter was measured at baseline. Stepwise hypotension (10 mm Hg per step) decreasing to 20 to 30 mm Hg was induced by controlled withdrawal of blood. At each step, systemic pressure, arteriolar inner diameter, CBF, and blood gases were measured 1 minute after the decrease in blood pressure. Ventilation (volume and rate) was adjusted as a function of inspired gases such that pH, PaO₂, and PaCO₂ were maintained within the physiological range. After the final stepwise fall in blood pressure, blood was reinjected and blood pressure returned to prehemorrhage values.

Vascular smooth muscle was fully relaxed by suffusion of cerebral vessels with artificial CSF that contained the calcium chelator EDTA (67 mmol/L), which produces complete deactivation of smooth muscle in cerebral arterioles. Pressure–internal diameter relationships were obtained in deactivated cerebral arterioles from a mean arterial pressure of 130 to 20 mm Hg by use of hemorrage to reduce pressure (steps of 10 mm Hg). At each pressure step, arteriolar diameter reached steady state within 15 seconds, and inner diameter was measured 30 seconds later. After the final step, blood was reinjected to restore blood pressure. Arterioles, maximally dilated by EDTA, were fixed at their physiological pressure in vivo by suffusion with glutaraldehyde (2.25% vol/vol, 0.10 mol/L cacodylate buffer). Arterioles were considered to be fixed adequately when blood flow ceased. After the animal was euthanized, the arteriolar segment was removed and embedded in paraffin.

The cross-sectional area (CSA) of the arteriolar wall was determined on 7-μm sections by use of the video image analyzing system described above. Luminal and total (lumen plus vessel wall) CSA of the arteriole were measured by tracing the luminal and outer edges of the vessel wall. CSA of the vessel wall was calculated by subtraction of luminal CSA from total CSA.

Calculations of CBF

Autoregulation Characteristics
Cerebral arteriolar inner diameter and blood flow values are reported as absolute values or as percentage change from baseline. For each group, CBF (absolute values and percentage of baseline), internal diameter (absolute values), arterial pressure, heart rate, and blood gas values were presented in the form used by Barry et al. Values were pooled and grouped by categories over mean arterial pressure ranges of 10 mm Hg. One-way ANOVA within these different mean arterial pressure ranges was performed for each treatment group. The lower limits of CBF autoregulation were defined as the lower limit of the lowest mean arterial blood pressure range in which CBF was not significantly less than baseline CBF. The security margin (percent- age), which indicates the degree to which mean arterial pressure may fall before CBF starts to decrease, was calculated as [(baseline mean arterial blood pressure−lower limit of CBF autoregulation)×100]/ baseline mean arterial blood pressure.

Calculation of Mechanical Characteristics

The assumptions on which we based the calculations of circumferential stress and strain and tangential elastic modulus have been described in detail previously. However, note that in the present experiments, systemic arterial pressure was used and not pial arteriolar pressure as previously described. The rationale for this approach is based on published data that show a linear relationship between systemic pressure (x) and pial arteriolar pressure (y) in cats. Furthermore, similar linear relationships were also found in SHR and WKY. Finally, the stress-strain curves that we obtained in the present experiments were similar to those obtained with pial arteriolar pressure measurements.

Circumferential stress σ at each pressure step was calculated from systemic arterial pressure P, inner diameter of cerebral arterioles Dᵢ, and wall thickness WT: σ = (P × Dᵢ)/2WT. Systemic arterial pressure was converted from millimeters of Hg to newtons per meter squared (1.334 × 10⁴). On the basis of the assumption that the volume of the vessel wall does not change in vessel diameter and pressure and on the previous finding that changes in vessel length during reductions in pressure are small, wall thickness was calculated from CSA of the vessel wall and cerebral arteriolar internal diameter: WT = [(4CSAπr + Dᵢ)²/2 − Dᵢ²]/2. External diameter Dₑ was calculated as Dₑ = Dᵢ + 2WT. Histological determinations of CSA were used in all calculations of wall thickness and circumferential stress. Circumferential strain ε was calculated as ε = (Dₑ − Dᵢ)/Dₑ, where Dᵢ is internal diameter at 20 mm Hg of pressure.

To obtain the tangential elastic modulus, the stress-strain data from each animal were fitted to an exponential equation (γ = aeᵇ) by use of least-squares analysis: σ = σₑeᵇ, where σₑ is stress at the pressure step of 20 to 29 mm Hg and β defines the rate of increase of the stress-strain curve. Tangential elastic modulus E₉ was calculated as E₉ = dσ/dε = βaeᵇ.

Substances Used
Gallamine triethiodide was purchased from Sigma Chemical Co, N₂ from Air Liquide, sodium pentobarbital from Sanofi Santé Animale, and KCl, MgCl₂, CaCl₂, NaCl, NaHCO₃, urea, and glucose from Merck KGaA. Lovastatin was a gift from MSD Research Laboratories (Paris, France).

Statistical Analysis
Results are expressed as mean±SEM. The experimental protocol was designed to use 1-way ANOVA with the variable “group” (WKY, SHR untreated, and SHR treated with lovastatin). Significant differences between means were determined with the Bonferroni test. One-way ANOVA with the variable “mean arterial blood
pressure range” was performed separately for each treatment group for the analysis of values obtained after hypotensive hemorrhage. The probability level chosen was \( P \leq 0.05 \).

**Results**

**Baseline Values**
Lovastatin significantly decreased mean arterial blood pressure in SHR (11 ± 4%), but pressure remained higher than in WKY. Heart rate, pH, and blood gases were similar in all groups. Lovastatin significantly increased internal diameter of cerebral arterioles before deactivation (21 ± 8%), but diameter remained smaller than in WKY. Lovastatin increased internal diameter of cerebral arterioles after deactivation to a level that was not significantly different to that observed in WKY and also normalized CSA (see Table).

**Vascular Mechanics**
After arterioles were deactivated, external diameter was significantly less in treated and untreated SHR than in WKY at mean arterial pressures of 130 to 20 mm Hg (Figure 1). The stress-strain curve in cerebral arterioles of untreated SHR was shifted to the right of the WKY curve (Figure 2), and the slope of tangential elastic modulus versus stress was significantly less in untreated SHR than in WKY (Table). Thus, passive distensibility was increased in cerebral arterioles of SHR, despite hypertrophic inward remodeling of the vessel wall. Lovastatin attenuated the rightward shift of the stress-strain curve (Figure 2) and the decrease in the slope of tangential elastic modulus versus stress (Table).

**Lower Limit of CBF, Security Margin, and Dilatation of Cerebral Arterioles**
After hypotensive hemorrhage in WKY, CBF remained constant until the 50 to 59 mm Hg pressure range and then significantly decreased; the lower limit of CBF autoregulation was 50 mm Hg, and the security margin, which indicates

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**Table 1: Baseline Values in WKY, Untreated SHR, and SHR Treated With Lovastatin**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WKY</th>
<th>SHR</th>
<th>SHR-Statin</th>
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<td><strong>Baseline measurements</strong></td>
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<td></td>
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<tr>
<td>Body wt, g</td>
<td>338±8</td>
<td>356±6</td>
<td>372±9*</td>
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<td>Systemic arterial pressure, mm Hg</td>
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<td>201±5*</td>
<td>181±3†</td>
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<td>Mean</td>
<td>106±3</td>
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<td>153±3†</td>
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<tr>
<td>Pulse</td>
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<td>52±3</td>
<td>48±3</td>
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<tr>
<td>Heart rate, bpm</td>
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<td>350±7</td>
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<td>Arterial blood gases, mm Hg</td>
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<td>( \text{Paco}_2 )</td>
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<td>38±0</td>
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<td>( \text{PaO}_2 )</td>
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<td>Cerebral arterioles before deactivation, ( \mu ) m</td>
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<td>Internal diameter</td>
<td>62±3</td>
<td>42±3*</td>
<td>51±2†</td>
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<td>Cerebral arterioles after deactivation</td>
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<td>Internal diameter, ( \mu ) m</td>
<td>108±5</td>
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<td>90±3</td>
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<tr>
<td>External diameter, ( \mu ) m</td>
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<td>96±3</td>
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<tr>
<td>Wall CSA, ( \mu ) m²</td>
<td>774±29</td>
<td>1,099±16*</td>
<td>826±52†</td>
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<tr>
<td>( E ) vs stress</td>
<td>7.1±0.9</td>
<td>4.5±0.4*</td>
<td>7.0±1.0</td>
</tr>
</tbody>
</table>

Internal diameter before deactivation of smooth muscle was measured at baseline arterial pressure. Internal diameter after deactivation of smooth muscle by EDTA was measured at a mean systemic arterial pressure of 100 to 109 mm Hg. External diameter after deactivation was calculated from measurements of internal diameter at 100 to 109 mm Hg mean pressure and histological measurements of wall CSA. \( E \) vs stress is given as the slope of \( E \) vs stress. Values are mean±SEM in 8 WKY, 9 untreated SHR, and 12 SHR treated with lovastatin (20 mg · kg \(^{-1}\) · d \(^{-1}\)).

\*\( P \leq 0.05 \) vs WKY; †\( P \leq 0.05 \) vs untreated SHR.

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**Figure 1.** Pressure–external diameter relationship in arterioles during maximal dilatation with EDTA (67 mmol/L) in untreated WKY (\( \bullet \); \( n=8 \)) and SHR that were untreated (\( \bigcirc \); \( n=9 \)) or treated with lovastatin (\( \triangle \); \( n=12 \)). Values are mean±SEM. \*\( P \leq 0.05 \) vs WKY.
the degree to which mean arterial pressure may fall before CBF starts to decrease, was 53% (Figure 3). The lower limit of CBF autoregulation was shifted to the higher mean arterial blood pressure values of 90 mm Hg in untreated SHR and 80 mm Hg in treated SHR, but the security margin remained constant (47% and 48%, respectively) (Figure 3).

In WKY, cerebral arterioles dilated significantly at pressures >50 mm Hg; maximal dilatation (38 ± 3%) was observed at 20 to 29 mm Hg. In SHR, cerebral arterioles dilated significantly at pressures <70 mm Hg and maximal dilatation (40 ± 4%) was observed at 40 to 49 mm Hg. In SHR treated with lovastatin, cerebral arterioles dilated significantly at pressures <60 mm Hg and maximal dilatation (36 ± 4%) was observed at 30 to 39 mm Hg. In SHR, the internal diameter was significantly less than in WKY at each pressure step (Figure 4).

Discussion

Chronic hypertension modifies the responses of cerebral blood vessels to acute decreases in pressure,19 which shifts

the lower limit of CBF autoregulation toward higher levels of blood pressure.20,21 Such altered vascular responsiveness may be due in part to hypertrophic inward remodeling of the vessel wall, which encroaches on the vascular lumen and reduces internal diameter.22–24 The reduction in internal diameter is also due to eutrophic inward remodeling, defined as a decrease in external diameter with no reduction in vascular passive distensibility.25 Inward remodeling allows the cerebral circulation to adapt to the higher blood pressure level and protects the brain against stroke. After treatment of hypertension, if the reduction in blood pressure is not accompanied by an increase in maximal vasodilation capacity, the risk of cerebral ischemia will increase.26 In the present study, lovastatin decreased hypertrophic inward remodeling of the vessel wall and attenuated the increase in passive distensibility in SHR but had no effect on eutrophic inward remodeling or CBF autoregulation.

Remodeling

Although pressure is an important determinant of hypertrophic inward remodeling,18,27 in the present study, lovastatin reduced hypertrophic inward remodeling but had little effect on systemic blood pressure. At least 3 hypotheses could explain this apparent discrepancy. First, although a close correlation exists between systemic and arteriolar pressures in normotensive and hypertensive models (see Methods), this may not be the case after treatment. We cannot rule out the possibility that lovastatin, even if it has a small but significant effect on systemic pressure, does not normalize mean or pulse pial arteriolar pressure. Previous work showed that a minor fall in pulse arteriolar blood pressure was accompanied by a major change in CBF autoregulation28 and in wall hypertrophy.12 Second, HMG-CoA reductase inhibitors such as lovastatin inhibit isoprenoid synthesis and prenylation of key cellular proliferation proteins such as members of the ras and rho families.29–31 Thus, they may modify CSA of the vessel wall independent of any change in blood pressure. Finally, HMG-CoA reductase inhibitors enhance eNOS activity.32 We have previously reported that NO may reduce hypertrophic inward remodeling in cerebral arterioles in hypertensive rats.7

Figure 3. CBF autoregulation during stepwise hypotension in untreated WKY (•; n=8) and SHR that were untreated (○; n=9) or treated with lovastatin (▲; n=12). CBF values (baseline percentage ± SEM) are grouped by mean arterial blood pressure ranges of 10 mm Hg (20 to 29 mm Hg to 190 to 199 mm Hg).10,11 Arrows show the lower limit of CBF autoregulation. *P <0.05 vs WKY.
Cerebral arterioles in SHR also undergo eutrophic inward remodeling; ie, a reduction in external diameter that cannot be attributed to a decrease in passive distensibility. Lovastatin did not significantly alter external diameter in SHR.

The prevention by lovastatin of hypertrophic inward remodeling and the nonsignificant effect on external diameter may be responsible for the small but significant increase in internal diameter that we observed before deactivation of cerebral arterioles in SHR treated with lovastatin.

During chronic hypertension in SHR, structural alterations of cerebral arterioles impair maximal diameter in deactivated arterioles and contribute to the increase in the lower limit of CBF autoregulation. A previous report found that hypertrophic inward remodeling accounts for only 25% of encroachment on the lumen, the remaining 75% being due to eutrophic inward remodeling. Thus, in the present experiment, although it prevented hypertrophic inward remodeling, lovastatin failed to decrease the lower limit of CBF autoregulation, because the treatment did not attenuate eutrophic inward remodeling.

**Vascular Distensibility**

Passive distensibility of cerebral arterioles increases in SHR, despite hypertrophic inward remodeling. This may be due to a reduction in the proportion of stiff (collagen and basement membrane) to compliant (smooth muscle, elastin, and endothelium) components. Therefore, lovastatin may attenuate the increase in passive distensibility by decreasing the proportion of smooth muscle cells and thus modify diameter measured after deactivation. A small (nonsignificant) effect was observed that could be linked to the effect of lovastatin on isoprenoid synthesis. However, the change in passive distensibility does not lead to any change in the lower limit of CBF autoregulation; this may not be the case for the upper limit of CBF autoregulation, which remains to be investigated.

**Conclusions and Implications**

Treatment with lovastatin in SHR prevented hypertrophic inward remodeling of the cerebral arteriole vessel wall and attenuated increases in passive distensibility but had no effect on cerebral arteriolar eutrophic inward remodeling. Given that eutrophic inward remodeling is the main determinant of cerebral vasodilatation, the lower limit of CBF autoregulation was not modified by lovastatin, and despite marked reduction in hypertrophic inward remodeling, the cerebral circulation of SHR treated with lovastatin remained well adapted to the high level of blood pressure.

The benefit of the treatment with lovastatin is that although it prevented arteriolar wall hypertrophy, it did not modify the way in which the cerebral circulation adapts to high blood pressure. This effect is probably due to its lack of effect on eutrophic inward remodeling. Furthermore, at the present time, we have no information on the effects of longer treatment withlovastatin, on the effects of an association lovastatin plus an antihypertensive drug, or on the effect oflovastatin on carotid artery distensibility and its subsequent effect on the cerebral circulation.

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**References**


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