Reduction of Cerebral Infarction in Stroke-Prone Spontaneously Hypertensive Rats by Statins Associated With Amelioration of Oxidative Stress

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Background and Purpose—This study aimed to clarify the effect of statins on spontaneous stroke and to examine the antioxidative effect in artificial transient middle cerebral artery occlusion (tMCAO).

Methods—Stroke-prone spontaneous hypertensive rats (SHR-SP) were treated with pitavastatin, atorvastatin, simvastatin, or vehicle for 4 weeks. Physiological parameters, serum lipids, and infarct volumes were examined. The markers for oxidative stresses on lipids and DNA were immunohistochemically detected in vehicle-treated or simvastatin-treated SHR-SP with tMCAO.

Results—Atorvastatin and simvastatin decreased infarct volumes, with simvastatin most effective. Simvastatin significantly reduced immunoreactivities for oxidative stress markers for lipids and DNA in neurons after tMCAO.

Conclusions—The results suggest that the antioxidative properties of statins may be implicated in their beneficial effects against neuronal damage in cerebral ischemia. (Stroke. 2005;36:670-672.)

Key Words: cerebral infarction ■ HMG-CoA reductase inhibitors ■ oxidative stress ■ rats, inbred SHR

Statins reduce cerebrovascular events by their pleiotropic effects independent of cholesterol-lowering mechanism.1 Antioxidative effects of statins have recently been noted as one of their pleiotropic effects. However, there have been few in vivo studies on statins showing their antioxidative effect in brain.2 Therefore, we first investigated the effect of statins on spontaneous stroke incidence using stroke-prone spontaneously hypertensive rats (SHR-SP). The antioxidative effect was then examined in an artificial occlusion model using oxidative stress markers for lipids and DNA.

Materials and Methods

Experimental Model

SHR-SP (Disease Model Cooperative Research Association, Kyoto, Japan) were divided into 4 groups; vehicle-treated (0.5% methyl cellulose in saline), pitavastatin-treated (10 mg/kg), atorvastatin-treated (20 mg/kg), and simvastatin-treated (20 mg/kg) groups (n=5 each group). Animals were fed ad libitum feed and 1% NaCl water. Statins were given orally for 28 days starting from age 10 weeks. Body weight and blood pressure were measured weekly. At 14 days, serum levels of triglyceride, total cholesterol, high-density lipoprotein cholesterol were measured. When stroke-like symptoms developed, the animals were euthanized. The animals with no symptoms were euthanized at 29 days. All animals were euthanized with intraperitoneal injection of an overdose of pentobarbital, and brains were quickly removed.

Because simvastatin most effectively reduced infarction volumes, SHR-SP (8 weeks old) were treated with simvastatin (n=12) or vehicle (n=10) for 14 days and then underwent 90 minutes of transient middle cerebral artery occlusion (tMCAO).3 At 4 or 24 hours after the reperfusion, animals (n=5 to 7, each time point) were euthanized. Frozen brains sections were stained with hematoxylin and eosin or cresyl-violet.

All experimental procedures were approved by the Animals Committee of the Graduate School of Medicine and Dentistry, Okayama University.

Measurement of Infarct Volume

For evaluation of the infarct area of spontaneous stroke, a set of sections was immunohistochemically examined for microtubule-associated protein 2.4 Areas devoid of microtubule-associated protein 2 staining (1:500; MAB364; Chemicon, Temecula, Calif) were regarded as infarcted regions. The infarct volume of each animal was calculated by summing the infarct areas in 5 sections (National Institutes of Health Image, version 1.62a).

Immunohistochemistry for N-(Hexanonyl)Lysine, 4-Hydroxynonenal, and 8-Hydroxy-2'-Deoxyguanosine

Immunoreactivities for N-(hexanonyl)lysine (HEL),4 4-hydroxynonenal (4-HNE), and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in tMCAO models were detected using antibodies against HEL (1:500; MHL-020; JAICA, Shizuoka, Japan), 4-HNE (1:400; MHN-020; JAICA), and 8-OHdG (1:50; MOG-020; JAICA).5 The numbers of positive cells for each marker in the 3 randomized 0.5 mm² areas in the ischemic core were counted.

Statistical Analysis

All data are presented as mean±SD. Statistical analyses were performed using Kruskal–Wallis H test and post-hoc Mann–Whitney U test for infarct volume and physiological and biochemical parameters. For positive cell numbers, Student t test was performed. P<0.05 was considered significant.
Physiological and Biochemical Parameters in SHR-SP
Blood pressure, body weights, and survival times were not different among the 4 groups during the treatment. The serum triglyceride level at 14 days was significantly decreased in the pitavastatin-treated group ($P$<0.05 versus vehicle-treated group) and tended to be lower in the atorvastatin-treated and simvastatin-treated groups compared with vehicle treatment. Statins did not affect the cholesterol levels.

Infarct Volume in SHR-SP
The infarct volumes in the vehicle-treated, pitavastatin-treated, atorvastatin-treated, and simvastatin-treated groups (each $n=5$) were 8.7±3.7, 4.4±5.2, 3.9±3.2, and 3.3±1.1 mm$^3$, respectively. Mann–Whitney $U$ test showed that atorvastatin ($P$<0.05) and simvastatin ($P$<0.01) significantly decreased infarct volumes compared with vehicle, although Kruskal–Wallis $H$ test did not reach statistical significance ($P$=0.11). No hemorrhage was detected in any animals.

Immunohistochemical Findings in tMCAO Animals
In simvastatin-treated animals, the intensity of staining for HEL, 4-HNE, or 8-OHdG was fainter than vehicle-treated ones (Figure A). Furthermore, simvastatin significantly decreased the number of positive cells for these oxidative stress markers (Figure B).

Discussion
This in vivo study demonstrated that chronic administration of statins reduced infarct volume in SHR-SP regardless of blood pressure and serum cholesterol levels, and that treatment with simvastatin ameliorated the oxidative stress on neurons in infarct area in artificial stroke.

We found that simvastatin was most effective against spontaneous stroke among 3 statins examined. All statins used are lipophilic, with simvastatin being the most lipophilic. Its strong liposolubility may result in high permeability through the blood–brain barrier to the parenchyma. Because small lesions at different stages of stroke were observed in SHR-SP, oxidative neuronal damage cannot simply be compared among the 4 groups in the spontaneous stroke model. Thus, we examined the antioxidative effect of simvastatin in tMCAO model and found substantial effects.

In the brain during and after ischemia, reactive oxygen species (ROS) impair the cell membrane by peroxidation of lipid bilayer and injure DNA, leading to cell death. Statins were shown to have antioxidant properties such as scavenging ROS, inhibiting ROS-induced DNA breakage, and restraining superoxide generation in vessel. Kawashima et al

| Physiological and Biochemical Parameters in SHR-SP |
|-----------------|--------|--------|--------|--------|
|                  | Vehicle ($n$=5) | Pitavastatin ($n$=5) | Atorvastatin ($n$=5) | Simvastatin ($n$=5) |
| Survival time (d) | 26.2±1.9 | 27.2±4.0 | 24.0±3.7 | 26.0±2.2 |
| Systolic BP (mm Hg) | 226.4±53.3 | 200.4±13.3 | 207.0±21.9 | 216.0±30.9 |
| Diastolic BP (mm Hg) | 171.4±24.5 | 154.2±4.8 | 169.0±26.2 | 165.0±22.4 |
| Body weight (g) | 233.6±15.8 | 231.2±16.6 | 238.0±19.7 | 243.0±9.9 |
| TG (mg/dL) | 111.2±24.3 | 67.8±19.2* | 90.2±22.9 | 91.8±22.1 |
| T-cho (mg/dL) | 69.6±10.2 | 70.2±6.5 | 67.8±6.3 | 78.2±4.5 |
| HDL-cho (mg/dL) | 35.0±2.6 | 33.6±2.9 | 34.2±4.2 | 38.2±1.9 |
| LDL-cho (mg/dL) | 7.0±2.0 | 6.6±1.5 | 6.5±0.6 | 8.4±0.9 |

* $P$<0.05 versus vehicle-treated group.
reported reduction of superoxide production in SHR-SP brain (nonstroke lesion) treated with cerivastatin.2 Our results suggest that statins protect the neurons against ROS-induced lipid peroxidation and DNA oxidation in the ischemic model.

Oxidative stress is profoundly involved in the pathophysiology of stroke. From the results of the present study, we believe that reduction of infarct volumes in statin-treated spontaneous stroke animals is associated with their antioxidative effect.

Acknowledgments
We thank for Banyu Pharmaceutical Co, Ltd, Kowa Company Ltd, and Yamanouchi Pharmaceutical Co, Ltd for their kind gifts of medicines used in this study. This work was partly supported by grant-in-aid for Scientific Research (B) 15390273 and (Hoga) 15659338, and National Project on Protein Structural and Functional Analyses from the Ministry of Education, Science, Culture, and Sports of Japan, by grants (Y. Itoyama, I. Kimura, and S. Kuzuhara) from the Ministry of Health and Welfare of Japan, and by grants from the Kanae Foundation for Life and Socio-Medical Science.

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*Stroke*. 2005;36:670-672; originally published online February 3, 2005;
doi: 10.1161/01.STR.0000155732.27333.3c

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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