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Research report

HMG CoA reductase inhibitors reduce ischemic brain injury of Wistar rats through decreasing oxidative stress on neurons

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Abstract

Statins possess neuroprotective effect against ischemic damage, but how they protect neurons is not exactly made clear. We speculated that anti-oxidative property of statins is implicated, and investigated statins' influences on the oxidative neuronal damage in the brain after ischemia. After 14 days of atorvastatin, pitavastatin, simvastatin, or vehicle administration, 90 min of middle cerebral artery occlusion was imposed on Wistar rats. The production of 4-hydroxynonenal (HNE) and 8-hydroxy-2′-deoxyguanosine (8-OHdG), both of which are oxidative stress markers, as well as infarction formation were investigated at 1 day after the reperfusion. In the vehicle group, massive infarction was confirmed and HNE and 8-OHdG are robustly produced. In the statins-treated group, the infarction was smaller and the HNE and 8-OHdG production was less prominent than the vehicle group. Among the statins investigated, simvastatin was most effective for reducing oxidative stress and infarction volume, which may be brought by its highly lipophilic property. Reduction of oxidative stress by statins may be one main reason in ameliorating ischemic brain damage in rats.
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Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: Cell death; Oxidative stress; Rat; Statin

1. Introduction

Reactive oxygen species (ROS) are implicated in neuronal cell death of various situations. They play quite important roles in the brain after ischemia and reperfusion in particular because a number of events that predispose the formation of ROS occur [6]. Many studies have indeed revealed that scavenging of ROS ameliorated the ischemic brain damage (reviewed by Lewen et al. [23]). Recently, the ROS scavenger became clinically available and is widely used for treating acute ischemic stroke [32]. In the chronic stage of stroke or for a preventive purpose, however, anti-oxidative treatment is not widely accepted.

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitors, referred to as statins, are widely prescribed for lowering serum cholesterol level [9]. As serum cholesterol is implicated in the atherosclerotic plaque formation [31,36], daily administration of statins should decrease the eventual risk of stroke and other vascular events. Actually, many analyses have demonstrated that statins decreased the risk of vascular events including ischemic stroke (reviewed by Hebert et al. [17]). In these studies, however, the beneficial effect of statins was more pronounced than expected by the

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; CBF, cerebral blood flow; eNOS, endothelial nitric oxide synthase; HDL-cho, high-density lipoprotein-cholesterol; HMG CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; HNE, 4-hydoxynonenal; LDL, low-density lipoprotein; MCA, middle cerebral artery; PBS, phosphate-buffered saline; rCBF, regional cerebral blood flow; ROS, reactive oxygen species; SODs, superoxide dismutases; T-cho, total cholesterol; TG, triglyceride

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cholesterol reduction [1,28]. Statins should thus possess primary preventive effects of vascular events or cytoprotective properties other than simple secondary effects by cholesterol reduction.

In ex vivo and in vitro studies, statins have anti-oxidative effects [2,34]. For example, copper- or leukocyte-induced oxidation of low-density lipoprotein (LDL) was inhibited by statins [18]. In addition, statins preserved superoxide dismutase activity, which is responsible for reducing oxidative stress [7]. Statins' beneficial effects for cerebrovascular disorders may thus be related also with its anti-oxidative property. In order to confirm that daily anti-oxidative medication through statin administration is implicated in its beneficial effects for stroke, we observed the change in neuronal damage and oxidative stress by daily statin administration using rat middle cerebral artery (MCA) occlusion model. As the oxidative stress marker, we used 4-hydroxynonenal (HNE)-conjugated proteins and 8-hydroxy-2′-deoxyguanosine (8-OHdG). HNE is an aldehyde produced by lipid peroxidation [12,16], which easily conjugates with various proteins. 8-OHdG is a product of nucleic acid oxidation [12], which is massively produced in the brain by ischemia and reperfusion [15].

2. Materials and methods

All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Okayama University's Administrative Panel on Laboratory Animal Care.

Male Wistar rats (10 weeks old, 230 g) were kept under diurnal lightning conditions and were allowed for food and water ad libitum. The animals were divided into four groups; 20 mg/kg of atorvastatin, 10 mg/kg of pitavastatin, or 20 mg/kg of simvastatin suspended in 0.5% methyl cellulose was perorally administered every day for each group animals (n = 6 or 7). For the vehicle group, 0.5% methyl cellulose was administered (n = 7). During this period, body weight of each animal was measured on alternative days.

Fourteen days later, the animals were anesthetized and ischemic insult was imposed. Under the anesthesia of a 69.5%/30% (vol/vol) mixture of nitrous oxide/oxygen and 0.5% halothane, a midline neck incision was made and the right common carotid artery was exposed. With use of a nylon thread, the right MCA was occluded through the common carotid artery, as our previous report [13]. In sham-control animals, the common carotid artery was exposed but MCA was not occluded. Before and during MCA occlusion, the regional cerebral blood flow (rCBF) was monitored by laser-Doppler probe, and the change in rCBF was calculated as percentage of normal condition [26]. During the operative procedure, body temperature was monitored and maintained at 37 ± 0.3 °C with a heating pad. After 90 min of transient ischemic period, cerebral blood flow (CBF) was restored by removal of the nylon thread under light anesthesia with diethyl ether. Then, the surgical incision was closed and the animals were allowed free access to water and food at ambient temperature (21–23 °C).

At 24 h after CBF restoration, the animals were anesthetized and blood samples were collected. Triglyceride (TG), total cholesterol (T-cho), high-density lipoprotein-cholesterol (HDL-cho), and low-density lipoprotein-cholesterol (LDL-cho) were measured using these samples. Just after blood collection, the animals were decapitated and the brains were removed. As ROS are robustly produced in the early time after reperfusion [6,23], we considered that the infarction volume at this time point reflects eventual brain damage. The brains were then quickly frozen, and sections of 10 μm thickness were prepared using a cryostat.

Sections from every 200 μm distance were stained by cresyl violet, and the infarction volume of each brain was calculated as previously reported [26]. The statistical significance of difference in infarction volume was also evaluated as reported [26].

For the immunohistochemical analysis, the sections were immersed in ice-cold acetone and well dried. They were then treated with 30% methanol and 0.3% hydrogen peroxide in phosphate-buffered saline (PBS) in order to quench the endogenous peroxidase activity. After blocking non-specific binding with 5% bovine serum albumin, the slides were incubated with primary antibodies at 4 °C for 10 h. The antibodies used and each dilution were mouse monoclonal anti-HNE antibody (MHN-20, JICA, Shizuoka, Japan) at 1:50 and mouse monoclonal anti-8-OHdG antibody (MOG-20, JICA) at 1:50.The slides were then incubated with biotinylated anti-mouse IgG (BA-2000, Vector Laboratories, Burlingame, CA) at 1:200 dilution in PBS. They were subsequently incubated with avidin–biotin–horseradish peroxidase complex (PK-6100, Vector Laboratories) and developed using diaminobenzidine as a color substrate. A set of sections was stained in a similar way without the first antibody. In these immunohistochemical studies, the positively stained cells at the MCA boundary zone cortex were counted and evaluated quantitatively as previously reported [14]. The statistical significance of difference in number of positively stained cells was also evaluated as reported [14].

Based on the results of above studies, we investigated neurological deficit of rats with vehicle or simvastatin administration. At 24 h after the reperfusion, neurological finding of each animal was scored as follows: 0, no neurological deficit; 1, difficulty to fully extend the left forepaw; 2, inability to extend the left forepaw; 3, circling to left side; 4, leaning to left. The results were analyzed and evaluated as previously reported [26].

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