Effect of Spinally Administered Simvastatin on the Formalin-Induced Nociceptive Response in Mice

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Received January 6, 2012; Accepted March 12, 2012

Abstract. Clinical and experimental observations indicated that 3-hydroxy-3-methylglutaryl CoA reductase inhibitor statins have pleiotropic effects. The present study determined the antinociceptive property of centrally administered simvastatin on the formalin-induced nociception in the mouse. Intrathecal administration of simvastatin at doses of 0.5 – 50 nmol dose-dependently attenuated the second, but not the first, phase of the formalin-induced nociception, which was partially reversed by mevalonate (5 μmol). Intracerebroventricular injection of simvastatin (50 nmol) did not affect the formalin-induced nociception. These results suggest that simvastatin-induced antinociception is mediated by attenuation of the sensitization of spinal nociceptive transmission.

Keywords: simvastatin, spinal cord, inflammation

Statins are widely prescribed for treatment of hyperlipidemia. Statins lower plasma cholesterol levels through the inhibition of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase. Several large clinical trials indicated that statins reduce the morbidity and mortality of coronary artery disease and the incidence of stroke (1). These pleiotropic effects of statins are considered to result from their actions other than lipid lowering, since the overall clinical benefits of statin therapy appeared to be greater than what might be expected from the lipid lowering effect (2). Indeed, another lipid lowering compound, ezetimibe, that inhibits intestinal cholesterol absorption did not show pleiotropic effects in humans (3).

It was reported that statins showed antinociceptive and antihyperalgesic effects in mice and rats (4, 5). Simvastatin and rosuvastatin systemic treatment also attenuated thermal hyperalgesia and mechanical allodynia in sciatic nerve–ligated rats (6). These effects of statins have been considered to result from their anti-inflammatory actions. Recently, we observed that spinal activation of the mevalonate pathway induced the thermal hyperalgesia through the activation of spinal RhoA/Rho kinase signaling (7). Therefore, it can be speculated that the statin-induced antinociception and antihyperalgesia is mediated by the inhibition of the mevalonate pathway in the central nervous systems. The present study was undertaken to define the action site of the highly lipophilic statin simvastatin on the formalin-induced nociceptive response in the mouse.

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Kyushu University of Health and Welfare, as adopted by the Committee on Animal Research of Kyushu University of Health and Welfare, which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Male ICR mice (Kyudo Laboratory Animals, Inc., Saga), weighing 20 – 30 g, were used in this study. Animals were housed five per cage in a room maintained at 23°C ± 0.5°C with an alternating 12-h light-dark cycle. Food and water were available ad libitum. Animals were used only once in all experiments.

The formalin test was performed according to the method described previously (7). After a 10-min acclimation period to individual observation cages, 25 μl of a 1.5% formalin solution was injected subcutaneously into
the dorsal aspect of the right hind paw and mice were then returned to the clear observation cages. The nociceptive response was recorded for 30 min. The mouse licked and bit the injected paw, and these responses were distinct and easily observed. The accumulated response time (seconds), that is, the duration of licking and biting of the injected paw, was measured for each 5-min block.

Intracerebroventricular administration was performed following the method described previously (8) using a 10-μl Hamilton syringe. The injection site was 1.5 mm from the midline, 0 mm from the bregma, and 3.0 mm from the surface of the skull. Injection volumes for intracerebroventricular administration were 4 μl.

Intrathecal injection in a volume of 5 μl was performed according to the methods of Hylden and Wilcox (9). The mouse was manually restrained and a 30-gauge needle matted to a 25-μl Hamilton syringe was inserted between L5 and L6 of the mouse spinal column.

The drugs used in the present study were simvastatin (Wako Pure Chemical Co., Ltd., Tokyo) and mevalonate (mevalonolactone; Sigma, St Louis, MO, USA). Other reagents used in the present study were molecular biology grade. Simvastatin and mevalonate were dissolved in a vehicle solution of 90% sterile saline, 5% dimethylsulfoxide (DMSO), and 5% cremophore EL (Sigma). This vehicle solution was used as the control treatment for all experiments in the present study.

The data were expressed as the mean ± S.E.M. The statistical significance of differences between the groups was assessed with an analysis of variance (ANOVA) followed by Dunnett’s test (comparisons of dose-response study) or the Bonferroni/Dunn test (comparisons between multiple groups) using GraphPad Prism version 3.0 software. *P < 0.05 was considered significant.

Subcutaneous injection of formalin (25 μl, 1.5%) into the hind paw caused a biphasic response of licking or biting for the injected paw: the first phase started immediately after injection and lasted for about 4 – 5 min and the second phase began 10 min after injection and lasted about 30 min (Fig. 1A).

Intrathecal injection of simvastatin dose-dependently attenuated the second phase of the formalin-induced nociceptive response (Fig. 1B). The first phase of the formalin-induced nociceptive response was not affected by intrathecal treatment with simvastatin (Fig. 1: A and B). The attenuation of the second phase of the formalin-induced nociceptive response reached statistically significant at doses of 5.0 and 50 nmol (Fig. 1B). In contrast to intrathecal injection, intracerebroventricular injection of simvastatin (50 nmol) did not affect any phase of the formalin-induced nociceptive response (Fig. 1C). Since intracerebroventricular injection requires higher dose than intrathecal injection to produce pharmacological effects (10), the effect of higher dose of simvastatin (100 nmol) was examined. Simvastatin at the dose of 100 nmol caused non-specific effects such as sedation, scratching, circling behaviors.

Since simvastatin has been indicated to have an anti-inflammatory effect after oral administration, the paw edema after formalin injection was examined. The thickness of the hind paw after formalin administration was not changed in intrathecal pretreatment with vehicle (before formalin injection: 2.76 ± 0.044 mm, after formalin injection: 4.37 ± 0.088 mm) or simvastatin (before formalin injection: 2.78 ± 0.040 mm, after formalin injection: 4.49 ± 0.12 mm).

The attenuation of the second phase of the formalin-induced nociceptive response in intrathecally administered simvastatin (50 nmol) was significantly reversed when mevalonate (5 μmol) was injected along with simvastatin (Fig. 2). Intrathecal injection of 5 μmol of mevalonate alone did not affect the second phase of the formalin-induced nociceptive response, although the first phase of the formalin-induced nociceptive response was potentiated (Fig. 2).

The present study indicates that intrathecal, but not intracerebroventricular, administration of simvastatin attenuated the second phase of the formalin-induced nociceptive response, the attenuation of which was partially reversed by the concomitant treatment with mevalonate.

Injection of formalin into the hind paw of mice causes the biphasic nociceptive response consisting of immediate (first) and tonic (second) phases (11). The first phase of the formalin-induced nociceptive response is produced by the direct stimulation of nociceptors by formalin itself, whereas the second phase is mediated by inflammation and central sensitization of nociceptive neurons (11). Intrathecal, but not intracerebroventricular, treatment with simvastatin dose-dependently attenuated the second phase, but not the first phase, of the formalin-induced nociceptive response, indicating that simvastatin inhibits the onset of central sensitization in the spinal cord. Since the first phase of the formalin-induced nociceptive response was not affected, simvastatin did not act on the nociceptive perception. Taken together these results, simvastatin may attenuate the sensitization of spinal nociceptive transmission.

We observed that simvastatin-induced attenuation of the second phase of the formalin-induced nociception was significantly reversed by concomitant treatment with mevalonate. This result suggests that the effect of simvastatin on the nociceptive transmission is partly mediated by the reduction of isoprenoid synthesis, because simvastatin inhibits HMG-CoA reductase, a rate-limiting enzyme of the mevalonate pathway. We further observed...
Fig. 1. Effects of intrathecal (i.t.) pretreatment with various doses of simvastatin on time course (A) and total duration of the response during the first (0–10 min) and second (10–30 min) phases (B) of the formalin-induced nociceptive response in mice, and effects of intracerebroventricular (i.c.v.) pretreatment with various doses of simvastatin on the total duration of the response during the first (0–10 min) and second (10–30 min) phases in mice (C). Simvastatin was injected i.t. or i.c.v. 30 min before the injection of formalin. Data are expressed as the total time spent in flinching. Each point and column represents the mean with S.E.M. of 10–11 mice in each group. B) One-way ANOVA revealed that i.t. administration with simvastatin dose-dependently decreased the second phase of the formalin-induced nociceptive response (F3,41 = 8.117, *P < 0.05, **P < 0.01, compared to the respective vehicle-pretreated group (Dunnett’s test).
that intrathecal treatment with mevalonate increased the response time of the first phase of the formalin-induced nociception. The HMG-CoA reductase inhibitor compactin was shown to impair the development of long-term potentiation in the hippocampal slices (12). This effect of compactin was restored by the supplementation of mevalonate (12). Moreover, depolarization increased the protein isoprenylation followed by the geranylgeranyl transferase activation in cultured hippocampal neurons (13). We recently reported that spinal mevalonate treatment produced hyperalgesia through the increased isoprenylation of RhoA (7). In the light of these results, it is possible that formalin-induced direct stimulation of nociceptors causes the protein isoprenylation in sensory neurons, which could trigger the spinal nociceptive sensitization.

Several investigations revealed that repeated injection with HMG-CoA reductase inhibitors produce the antinociception in mice and rats. It is reported that systemic treatment with atorvastatin produces antinociceptive and antiinflammatory effects in inflammatory pain induced by bacterial adjuvant (4) or lipopolysaccharide (5). Repeated treatment with simvastatin and rosuvastatin also produces an antihyperalgesic effect in nerve injury–induced neuropathic pain in mice and rats (6). These reports suggest that these effects of HMG-CoA reductase inhibitors might be mediated by the anti-inflammatory effect. We did not observe the inhibitory effect of simvastatin on the formalin-induced hind paw edema. Therefore, it is possible that the antinociceptive effect of single injection of simvastatin is mediated by the attenuation of sensitization of spinal nociceptive transmission rather than the anti-inflammatory effect. The discrepancy between our results and previous observations might be due to the different treatment regimen, that is, single or repeated treatment. To support our hypothesis, there are reports indicating that HMG-CoA reductase inhibitors modulate nerve activity independent from their anti-inflammatory effects (14, 15).

In conclusion, our present data suggest that simvastatin-induced antinociception is mediated by the inhibition of the sensitization of spinal nociceptive transmission and that the antinociceptive effect is produced by the attenuation of mevalonate synthesis in the spinal cord. The neural mevalonate pathways play an important role for the sensitization of neurotransmission in the central nervous systems.

Acknowledgments

We thank Ms. Mari Oji for her technical assistance. This study was supported by the Ministry of Education, Culture, Sports, Science, and Technology, Grant-in-Aid for Young Scientist (B).
References