Neuroscience 167 (2010) 954–963

LOVASTATIN IMPROVES NEUROLOGICAL OUTCOME AFTER NUCLEUS BASALIS MAGNOCELLULARIS LESION IN RATS

Z. ZHAO,[a1] S. ZHAO,[b1] N. XU,[c1] C. YU,[d] S. GUAN,[e] X. LIU,[c] L. HUANG,[d] W. LIAO[d]* AND W. JIA[b,f]

[a]*College of Traditional Chinese Medicine, Hebei Medical University, Shijiazhuang, PR China*

[b]*PepMetric and Pegasus Pharmaceuticals Group Inc. Unit 110-116, 11800 River Road, Richmond BC, V6X 1Z7 Canada*

[c]*Department of Rheumatology and Immunology, Third Hospital of Peking University, Beijing 100029, PR China*

[d]*National Integrative Medicine Centre for Cardiovascular Diseases, China–Japan Friendship Hospital, Beijing, PR China*

[e]*Department of Pharmacology, Hebei Medical University, Shijiazhuang, PR China*

[f]*Department of Surgery, Brain Research Centre, University of British Columbia, 2211 Wesbrook Mall, Vancouver, V6T 2B5 Canada*

Abstract—Increasing evidence indicates that statins, specific inhibitors of 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase, exerts neuroprotective actions rather than simply lowering cholesterol. However, the underlying mechanism has not been elucidated clearly. Here, the effect of lovastatin on the neurological outcomes of nucleus basalis magnocellularis (NBM)-lesioned rats and the pathophysiological mechanisms were investigated. Sprague–Dawley rats were divided into three groups: (i) a sham group; (ii) a model group: bilateral NBM of rats were injured by infusion of ibotenic acid; and (iii) a lovastatin-treated group: lovastatin was administrated orally for 4 weeks before treated by ibotenic acid. We show that lovastatin significantly improves the neurological outcomes as well as the choline acetyltransferase (ChAT) activity and muscarinic/NMDA receptor binding activity impaired by NBM lesion, and that lovastatin prevents neuron loss and induces Akt whereas inhibits p38 phosphorylation. Overall, the neuro-restorative and -protective effect of lovastatin may be attributed to the regulation of Akt- and p38-mediated signaling pathway together with improvement of muscarinic/NMDA receptor functions. Statins may be useful in the treatment of neurological disorders.

Key words: lovastatin, Morris Water Maze, nucleus basalis magnocellularis, neuroprotection, muscarinic/NMDA receptor, signaling pathway.

Statins, specific inhibitors of 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase, are commonly used for the treatment of hypercholesterolemia (Kita et al., 1980; Dembowski and Davidson, 2009). However, accumulating evidences show that this group of medicines also exert beneficial effects in neurological disorders, such as Alzheimer's disease (AD), traumatic brain injury and stroke (Gluhovschi et al., 2008; Kandiah and Feldman, 2009; Gomis et al., in press). Brain cholesterol metabolism has been reported to closely link with the pathological process of AD, and the content of 24S-hydroxycholesterol, a detrimental polar metabolite of cholesterol, in cerebrospinal fluid (CSF) is significantly higher in AD and vascular demented patients at early stages of the disease (Lütjohann and von Bergmann, 2003; Evans et al., 2009). Statins can initially reduce cholesterol and 24(S)-hydroxycholesterol in CSF and play an important role in the maintenance of brain cholesterol homeostasis, and thus exert therapeutic potential in AD (Dolga et al., 2008; Kandiah and Feldman, 2009; Handattu et al., 2009). In addition, statins also can reduce delayed neuronal death, increase cerebral blood flow and neurogenesis and improve neurological disturbances after traumatic brain injury (Qu et al., 2005; Lu et al., 2007; Wang et al., 2007; Wu et al., 2008; Chen et al., 2009). These strongly suggest that statins are potential candidates for treatment of neurological disorders though its neuro-restorative and -protective actions.

Though increasing reports indicated that the main signaling pathway involved in these beneficial effects of statins is mediated by mitogen-activated protein kinase (MAPK) or Phosphoinositide-3 kinase (PI3K), or the both (Sironi et al., 2006; Cerezo-Guisado et al., 2007a,b; Rupérez et al., 2007; Wu et al., 2008; Dolga et al., 2008; Reiss and Wirkowski, 2009), more details of the mechanisms remain unclear. In addition, dysfunctions of both the cholinergic system, e.g. the decreased choline acetyltransferase activity, and of the muscarinic and N-methyl-D-aspartate (NMDA) receptors, are closely related to the neurological disorders, and improvement of these receptors' function can relieve the neuropathological process and improve the ability of learning and memory (Riedel et al., 2003; Contestabile et al., 2008; Mufson et al., 2008; Bois et al., 2009; Ho et al., 2009; Wang et al., 2008, 2009). To our knowledge, whether statins can regulate the functions of these receptors in brain and as a consequence exert neuro-restorative and -protective actions has not been clarified yet.

In the present study, the effects of lovastatin on the neurological outcomes of nucleus basalis magnocellularis (NBM)-lesioned rats induced by ibotenic acid was examined through Morris Water Maze test, and the binding activity of membrane receptors, the cholinergic system muscarinic receptor and glutamatergic NMDA receptor, is also determined after the treatment of lovastatin. Our data

[1] These authors contributed equally to this work.

*Corresponding author. Tel: +86-10-84205625; fax: +86-10-64417748.
E-mail address: lwqxy@hotmail.com (W. Liao).

Abbreviations: AD, Alzheimer's disease; ChAT, choline acetyltransferase; HE, Haematoxylin-Eosin; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; MAPK, mitogen-activated protein kinase; NBM, nucleus basalis magnocellularis; PI3K, Phosphoinositide-3 kinase; TAB, tris–acetate-buffer.

0306-4522/10 $ - see front matter
doi:10.1016/j.neuroscience.2010.02.054

show that lovastatin significantly improves the learning and memory ability as well as the choline acetyltransferase (ChAT) activity and muscarinic/NMDA receptor binding, and prevents neuron loss against detrimental factors probably involved in its regulation on p38- and Akt-involved signaling pathways, and make great contributions to a better understanding of the critical roles of statins in treating neurological disorders and to further clarify to the underlying mechanisms of statin-mediated neuro-protective and -restorative actions.

EXPERIMENTAL PROCEDURES

Animals

Fifty male Sprague–Dawley rats (180±15 g) were purchased from Health Science Center of Peking University. On the day of surgery, animals were randomly divided into three groups, with 16 rats in each group, as follows: (i) a sham-operated group; (ii) a NBM-lesioned model group: rats were treated by operation with bilateral NBM lesion; and (iii) a lovastatin-treated group: rats were treated by lovastatin (Sigma-Aldrich, St. Louis, MO, USA) plus NBM lesion. Through the experiment, animals were maintained in a room at constant temperature (24 °C) and under a 12 h light–dark cycle. This work was undertaken as required by the animal ethics committee of Peking University (Beijing, China), and was carried out in accordance with the USA NIH guidelines [Guide for the Care and Use of Laboratory Animals (1985), DHEW Publication no (NIH). 85-23: Office of Science and Health Reports, DRR/NIH, Bethesda, MD, USA].

Animal model

Rats in the sham and model group were administered orally with 0.5% carboxymethyl cellulose (CMC, Sigma-Aldrich, St. Louis, MO, USA) and rats in the lovastatin group with lovastatin 5 mg/kg/d (dissolved in 0.5% CMC) once a day for consecutive 4 weeks. On the operation day, all rats in each group were anaesthetized with 1% pentobarbital sodium (Sigma-Aldrich, St. Louis, MO, USA; 0.5 ml/100 g body weight, i.p. injection) and fixed in a stereotaxic frame. Ibotenic acid (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in pH 7.4 phosphate buffer saline at final concentration of 5 μg/μl and 1 μl was infused into both sides of NBM (A 1.0 mm; L±2.7 mm; V 7.8 mm) of rats in the model and lovastatin group for 3 min according to previous report (Paxinos and Watson, 1986), and PBS (1 μl) for rats in the sham group. All rats recovered well after surgery.

Morris Water Maze apparatus

The Morris Water Maze was a black circular stainless tank, measuring 1.40 m in diameter and 40 cm in depth. When doing experiment, the tank was filled with innocuity black dye water to 28 cm in depth, and a black platform located in a fixed quadrant is 26 cm in height, 8 cm in diameter, 30 cm away from the side wall, 2 cm below water surface, which is invisible for the animals. The apparatus was put in the middle of a sound-proof room (3.1×2.8 m^2).

Behavioral experiments

Ten days after lesion, Morris Water Maze test was started. During each of the five daily sessions, the animals performed three trials. A trial was started by placing the rat into the pool, facing the wall of the tank. The pool was divided in four imaginary quadrants (N–S–E–W) and during the whole training phase the platform was placed in the middle of the West quadrant (at 30 cm from the pool rim). Rat was placed to pool from one of the three quadrants other than the West one and was allowed to swim for a maximum of 90 s searching for the platform. If the animal found the platform during this time, it was left for a 10-s period on top of it. Otherwise, the animal was guided towards the platform and allowed a 30 s stay on it. There was a 10 min interval between two trials. Swimming patterns were recorded through a camera monitor with a computer using the Jiliang system (Shanghai JL Neuro-Behaviro Science Inc., China).

During the probe trial the platform was removed, and the time that a rat spent in the four quadrants was measured for 60 s. The area around the previous platform position was defined as the annulus region (diameter 30 cm). The time that animals spent in this region and the times of crossing through the previous platform position were recorded. All rats started from the same position, opposite to the quadrant where the escape platform had been positioned during acquisition. After completion of the last trial of each day the rats were dried and returned to their home cages. There the animals were kept warm for 5–10 min under an infrared bulb (Original Hanau Solilux, 150 W) fixed about 60 cm above the floor of the cage.

Haematoxylin-Eosin (HE), Nissl staining, and TUNEL assay

After completing the probe trial the rats were decapitated after injection of a lethal dose of chloral hydrate. The brains were rapidly removed and frozen in n-methylbutane at −40 °C. Horizontal sections were cut across the nucleus basal magnocellularis and the region of the hippocampus in the horizontal plane with a cryostat microtome. 20-μm sections were used for regular HE and Nissl staining. 6-μm sections were used for dUTP nick-end labeling (TUNEL) assay as previously described (Zhao et al., 2009). Three sections from each group were randomly selected, and ten microscopic fields per section were evaluated and the percentage of unhealthy (appeared as either indistinct, lacking a clear cell boundary, with a small darkened nucleus, or darkened, shrunken) or apoptotic cells was calculated.

Western blot analysis

Western blot was performed as described previously (Chen et al., 2009). After behavioral test, three rats in each group were sacrificed by decapitation and the part of hippocampus and frontal cortex were isolated and homogenized with lysis buffer. After protein concentration was determined using the BCA protein assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Protein (40 μg) was loaded onto a 10% SDS/polyacrylamide gel and electrophoretically transferred to nitrocellulose membranes (Pall Corporation, East Hill, NY, USA), immunoblotted with antibodies against phospho-p38 and phospho-Akt (Cell Signaling Tech., Beverly, MA, USA), and p38 and Akt (Santa Cruz Biotech. CA, USA) according to the supplier's protocol, and visualized with peroxidase and an enhanced-chemiluminescence system (ECL kit, Pierce Biotechnology, Inc.).

ChAT activity assay

After behavioral test, rats were sacrificed by decapitation and the hippocampus and frontal cortex were isolated and stored at −80 °C, separately. The activity of ChAT was determined by the radioenzymatic assay as described previously (Fonnum, 1975). For preparation of homogenates, hippocampus and front cortex were homogenized with 10-time volumes of ice-clold PBS with 10 mM EDTA, respectivelly. Protein concentration was calculated as above. 50 μl homogenate was incubated with 50 μl reaction solution (choline chlotide 10 mM, EDTA sodium 10 mM, NaCl 300 mM, physostigmine sulfate 1 mM, Albumin 0.25%) containing 0.1 nM [^{3}H]-Acetyl-CoA (684 GBq/mmol, NEN Life Science, Boston, MA, USA) at 30 °C for 30 min. The reaction was terminated by 3.0 ml ice-cold PBS and the radioactivity was measured by liquid scintillation spectrometry with a counting efficiency of 40%–42%.