Atorvastatin Pretreatment Attenuates Ischemic Brain Edema by Suppressing Aquaporin 4

Zhi-Juan Cheng, MD,* Ting-Min Dai, MD,† Yao-Yao Shen, MD,† Jian-Le He, MD,* Juan Li, MD,* and Jiang-Long Tu, PhD*

Background: Cerebral edema, a serious complication of acute cerebral infarction, has a crucial impact on morbidity and mortality in the early stage of cerebral infarction. And aquaporin 4 (AQP4), a bidirectional water transporting protein, plays a pivotal role in edema formation. At experimental model, it has proven that atorvastatin could exert pleiotropic neuroprotection on acute cerebral infarction independent of its cholesterol-lowering action. It was a common protective manifestation that atorvastatin can reduce the infarct volume and cerebral edema. However, little is known about atorvastatin improving ischemic brain edema by regulating AQP4 expression. This study intended to investigate the neuroprotection effects of atorvastatin pretreatment in rats with cerebral ischemia and further explore the potential relationship between atorvastatin and AQP4 expression. Methods: Fifty-one adult male Sprague Dawley rats were randomly divided into 3 groups: sham, middle cerebral artery occlusion (MCAO), and atorvastatin pretreatment (Ator) group. For Ator group, 20 mg/kg of atorvastatin injectable suspension was administered once for 7 days by gavage before operation, whereas the others were administered the same volume of saline matching. Except for sham group, MCAO and Ator groups were subjected to permanent MCAO by modified intraluminal suture method. Infarct volume, neurological deficit, brain water content (BWC), immunohistochemistry, western blot, and polymerase chain reaction (PCR) were measured at 24 hours after MCAO. Results: Compared with sham group, the mNSS, infarct volume, and BWC of ischemic hemisphere were significantly increased ($P < 0.001$) in MCAO group. Positive cells and protein levels of p-p38MAPK and AQP4 in peri-infarction were significantly increased ($P < 0.01$). The mRNA levels of p38MAPK and AQP4 were also prominently upregulated ($P < 0.01$). Interestingly, preadministration of atorvastatin dramatically decreased infarct volume and the BWC of ischemic hemisphere compared with MCAO group ($P < 0.05$). The overexpressions of p-p38MAPK and AQP4 in peri-infarction were significantly decreased ($P < 0.05$) and their mRNA levels were downregulated by atorvastatin pretreatment ($P < 0.05$). Neurological deficits were also dramatically improved ($P < 0.001$). Conclusion: To the best of our knowledge, this is the first study that demonstrates

From the *Department of Neurology, The Second Affiliated Hospital of Nanchang University, China; and †Department of Neurology, Affiliated Hospital of Jiujiang University, China.

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Address for correspondence to Jiang-Long Tu, Department of Neurology, The Second Affiliated Hospital of Nanchang University, 1 Minde Road, Nanchang, Jiangxi 330006, China. E-mail: 1219467692@qq.com 781235963@qq.com 852806529@qq.com 1509030036@qq.com 13576043156@qq.com tujianglong85@126.com

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an effect of atorvastatin on expression of AQP4, and we propose that decreased AQP4 expression through a p38MAPK-suppression pathway may be the mechanism of atorvastatin alleviating ischemic cerebral edema.

Keywords: Atorvastatin—cerebral ischemia—cerebral edema—aquaporin 4—phosphorylated-p38MAPK.

Introduction

Cerebral edema, abnormal accumulation of brain water, is a common complication of acute cerebral infarction and has a crucial impact on morbidity and mortality in stroke.[1-3] However, current treatments of cerebral edema are limited to conventional osmotic agents, such as mannitol and surgical decompression, none of which could correct the molecular-level mechanisms responsible for brain edema caused by large middle cerebral artery occlusion (MCAO) or massive hemispheric infarction.[4] Therefore, novel therapeutic strategies were necessary for brain edema prevention.

Aquaporin 4 (AQP4) as a bidirectional water transporting protein plays a pivotal role in cerebral water homeostasis by regulating water transport.[3-5] In addition, in the studies of permanent cerebral ischemia or ischemia reperfusion model, *AQP4* knockout mice can significantly reduce cerebral infarct volume and edema compared with wild-type mice.[4,5] Therefore, therapy of targeting AQP4 is a potential method for improving ischemic brain edema. Animal experiments have shown that some drugs, such as ulinastatin,[6] ghrelin,[7] 3-nitropropionic acid,[8] or p38MAPK inhibitor,[9] can improve ischemic cerebral edema by inhibition or downregulation of AQP4 expression, but none has been brought to clinical options.

Statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, are a widely used group of cholesterol-lowering agents with high efficacy and safety as well as good tolerance. In the stroke-prone spontaneously hypertensive rats model, it has demonstrated that long-term pretreatment with atorvastatin can prevent the prevalence of spontaneous stroke and delayed stroke death through a cholesterol-lowering independent mechanism.[10] In addition, statins also can exert pleiotropic neuroprotection on acute cerebral infarction by various pathways independent of their lipid-lowering action, such as isoprene, NF-κB, PI3K/Akt, MAPKs, and apoptosis pathway.[11] Moreover, atorvastatin is a lipophilic synthetic drug and can through blood-brain barrier (BBB),[12] which has become a front-line preventive and therapeutic drug and was widely used in both clinical trials and animal studies of cerebral ischemia. Although many researches have shown that atorvastatin could reduce the infarct volume and cerebral edema,[13-15] it is not clear that whether atorvastatin could improve ischemic brain edema by regulating AQP4 expression. In this study, we intended to investigate the effects of atorvastatin pretreatment on cerebral ischemia edema and AQP4 expression in rats with focal cerebral ischemia and further explore the possible correlation between atorvastatin and AQP4 expression.

Experimental Procedures

Drug Administration and Ischemia Protocol

Fifty-one male Sprague−Dawley rats (slack King animal experiment center, China) (200-250 g) were used and allowed to acclimatize for 5 days before the experiment began. All animals care and experimental procedures were carried out according to the NIH Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996) and the guidelines of the Animal Care and Use Committees of Nanchang University, China.

Rats were randomly divided into 3 groups (n = 17 in each group): sham, MCAO, and atorvastatin pretreatment (Ator) group. For Ator group, atorvastatin tablets (Pfizer, Dalian, China) 20 mg/kg[13] were comminuted and dissolved in 0.9% NaCl solution to prepare injectable suspension with concentration of 5 mg/ml and was administered once for 7 days by gavage before operation, whereas the others were administered the same volume of saline matching. Animals were fasted overnight before and after surgery and had free access to water.

Under chloral hydrate (350 mg/kg) intraperitoneal anesthesia, permanent MCAO (pMCAO) was carried out by modified intraluminal filament method.[16,17] Briefly, a 3-0 nylon monofilament with a round tip was inserted from the external carotid artery into the internal carotid artery until the distal end meets mild resistance, indicating occlusion of origin of middle cerebral artery was successful. MCAO were maintained for 24 hours. Sham group rats subjected to the same surgical procedures except for MCAO. 24 hours after operation of 3 groups, 1 rat was randomly selected from each group to verify that pretreatment atorvastatin could reduce the volume of cerebral infarction as predecessors described. And then, 4 rats were selected from each group for measuring neurological deficit, brain water content (BWC), immunohistochemistry, western blot, and PCR.

Neurological Function Assessment

A modified 18-point score[18] was used to assess neurological impairment to the experimental rats (n = 17 in