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BRAIN RESEARCH

Research Report

Neuroprotection of early and short-time applying atorvastatin in the acute phase of cerebral ischemia: Down-regulated 12/15-LOX, p38MAPK and cPLA2 expression, ameliorated BBB permeability

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ABSTRACT

Background: It has been proved that chronic administration and pre-treatment with atorvastatin could protect brain tissue against ischemic injury. However, little is known regarding the effect of atorvastatin in the acute phase of ischemic stroke. This study investigated the potential neuroprotective effects of atorvastatin and underlying mechanisms in vivo. *Methods:* Male Sprague–Dawley rats were subjected to permanent middle cerebral artery occlusion (MCAO). Experiment 1 was used to evaluate time course expressions of 12/15-LOX, mitogen-activated protein kinase (MAPK), phosphorylated-p38MAPK (phospho-p38MAPK) and cytosolic phospholipase A2 (cPLA2) after cerebral ischemia, seven time points were included. Experiment 2 was used to detect atorvastatin's neuroprotection in the acute phase of ischemic stroke; atorvastatin was administered immediately after MCAO. Neurological deficit, brain water content and infarct size were measured at 24 h after stoke. Immunohistochemistry, reverse transcription-polymerase chain reaction (RT-PCR) and Western blot were used to analyze the expression of 12/15-LOX, p38MAPK, phospho-p38MAPK and cPLA2. Experiment 3 was used to detect atorvastatin's influence on blood–brain barrier (BBB). *Results:* 12/15-LOX, p38MAPK, phospho-p38MAPK and cPLA2 were up-regulated after cerebral ischemia. Compared with MCAO group, atorvastatin dramatically reduced brain water content and infarct sizes, and the over-expressions of 12/15-LOX, p38MAPK, phospho-p38MAPK and cPLA2 were significantly decreased in high dose group (20 mg/kg, $P<0.05$). Meanwhile, extra-vascular IgG was not only reduced, but BBB permeability was also ameliorated. *Conclusions:* Atorvastatin protected brain from damage caused by MCAO at the early stage; this effect may be through down-regulation of 12/15-LOX, p38MAPK and cPLA2 expressions, and ameliorating BBB permeability.

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1. Introduction

Chronic administration and pre-treatment with statins can reduce infarct volume and improve neurological deficit in mouse models of cerebral ischemia (Amin-Hanjani et al., 2001; Nagotani et al., 2005; Yrjänheikki et al., 2005; Tanaka et al., 2007). On the contrary, withdrawal of these drugs in the acute phase may impair vascular function and cause a greater extension of infarct volume and poorer functional outcomes in the stage of recovery (Gertz et al., 2003; Blanco et al., 2007). However, there is still a paucity of data about the exact role of statins on the brain parenchymatous tissue in the acute phase of cerebral ischemia. Increasing evidences have shown that statins have pleiotropic protective actions that are independent of lipid-lowering effect (Inoue et al., 2000; Laufs et al., 2000; McGirt et al., 2002; Zacco et al., 2003). It had been reported that atorvastatin up-regulated endothelial nitric oxide synthase (eNOS) and type III nitric oxide synthase in thrombocytes, decreased platelet activation, and lessened cerebral damage induced by ischemia in normocholesterolemic mice (Laufs et al., 2000). In addition to effects on cerebrovascular function, atorvastatin has the potential to render cortical neurons more resistant to NMDA-induced excitotoxic death and oxidative damage induced by ischemia (Zacco et al., 2003; Nagotani et al., 2005). Moreover, atorvastatin also protected brain against inflammatory injuries and regulated the actions of inflammatory factors, such as, up-regulating interleukin-4 (IL-4) and peroxisome proliferator-activated receptor gamma (PPAR gamma), inhibiting the activation of interleukin-1beta (IL-1beta), matrix metalloproteinase 9 (MMP9), extracellular signal-regulated kinase (ERK) and NF-kappa B (Ye et al., 2006; Clarke et al., 2008). These findings lead to the hypothesis that atorvastatin might play an important role in inhibiting inflammatory injuries induced by ischemia.

Arachidonic acid (AA) metabolism is one of classical oxidative stress function ways (Muller and Sorrell, 1997; Pompeia et al., 2002; Nakamura et al., 2003). Lipoxygenases (LOXs) pathway is one of the major ways of AA metabolism. LOXs derivatives from AA, such as, 12- and 15-hydroxy/hydroperoxyeicosatetraenoic acids (12- and 15-HETE) and lipoxin A4 (LxA4), act as the second messengers to promote tissue injury and repair process (Sharma et al., 2005; Nagasawa et al., 2007; Sexton et al., 2007; Prasad et al., 2008). Release of AA induced by cytosolic phospholipase A2 (cPLA2) is the rate-limiting step in the 12/15-LOX pathway. Several reports have shown that 12/15-LOX derivatives from AA can directly activate p38 mitogen-activated protein kinase (p38MAPK) and stimulate its phosphorylation (Reddy et al., 2002), and phosphorylated p38MAPK (phospho-p38MAPK) is linked to activation and phosphorylation of cPLA2 and AA release (Nito et al., 2008). The interaction between 12/15-LOX and phospho-p38MAPK/cPLA2 pathway promoted the progression of AA metabolism, generated a series of lipid mediators, and exacerbated inflammatory process and tissue injury. In this study, we investigated whether there might be an interaction between atorvastatin and AA metabolism mediated by 12/15-LOX pathway so as to further identify atorvastatin's anti-inflammatory effects in the acute phase of ischemic stroke.

Blood–brain barrier (BBB) existing at brain microvessel endothelial cells (BMVECs) acts as an interface separating the brain parenchyma from the systemic circulation. Breakdown of the BBB is an early and prominent event in cerebral ischemia (Petito, 1979). Tight junctions are important structural components of the BBB, which are essential for maintenance of the BBB, including zonula occludens (ZOs), claudins and occludin (Mark and Davis, 2002). Among these tight junction proteins, the transmembrane protein claudins is critically involved in sealing the tight junctions, and BMVECs predominantly express claudin-5 (Morita et al., 1999). Disruption of claudin-5 alone is enough to cause functional changes of the tight junctions (Nitta et al., 2003). Kalayci et al. (2005) have demonstrated that atorvastatin attenuated BBB permeability through increasing ZO-1 and occludin. Thus, we investigated atorvastatin's effect on claudins.

2. Results

2.1. *12/15-LOX, p38MAPK and cPLA2 were up-regulated in cerebral ischemia*

Immunohistochemistry, Western blot and reverse transcription-polymerase chain reaction (RT-PCR) were used to detect the time course expressions of 12/15-LOX, p38MAPK, phospho-p38MAPK and cPLA2 in brain tissue at normal, 3, 6, 12, 24, 48, and 72 h after permanent occlusion of the middle cerebral artery (MCAO) (Fig. 1). Compared with normal-control group, the protein levels of phospho-p38MAPK and cPLA2, and the mRNA levels of p38MAPK and cPLA2 were up-regulated beginning at 3 h ($P<0.05$), getting to high values at 24 h and peaking at 48 h after MCAO ($P<0.05$). The result of immunohistochemistry of phospho-p38MAPK and cPLA2 was consistent with those of RT-PCR and Western blot. The expression of 12/15-LOX was up-regulated at 12 h ($P<0.05$), and got peak values at 48 h ($P<0.05$). All the results of immunohistochemistry, Western blot and RT-PCR showed that compared with 3, 6 and 12 h, the expressions of 12/15-LOX, phospho-p38MAPK and cPLA2 at 24 h after permanently MCAO were significantly increased ($P<0.05$), but slightly lower than peak values.

2.2. *Atorvastatin reduced the expressions of 12/15-LOX, phospho-p38MAPK and cPLA2 in the acute phase of ischemia*

The expressions of positive cells of 12/15-LOX, phospho-p38MAPK and cPLA2 were observed in ischemic cortex around infarct regions at 24 h post-ischemia before and after treatment with atorvastatin (Fig. 2A, B and C). Outcome of immunohistochemistry (Fig. 2A, B and C) showed that the number of positive cells of 12/15-LOX, phospho-p38MAPK and cPLA2 dramatically increased in ischemic cortex. High dose of atorvastatin significantly reduced the positive cells of 12/15-LOX, phospho-p38MAPK and cPLA2 after MCAO ($P<0.05$). In agreement with results of immunohistochemistry, Western blot (Fig. 2D–I) and RT-PCR (Fig. 3) analyses also showed a significant decrease of 12/15-LOX and cPLA2 in high dose group at both protein and mRNA levels ($P<0.05$). This marked reduction remained in Western blot for phospho-p38MAPK and in RT-PCR for p38MAPK in high dose group. However, there were no significant differences in the expressions of 12/15-LOX, phospho-p38MAPK and cPLA2 between MCAO group and low dose group ($P>0.05$).