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Statins excert neuroprotection on cerebral ischemia independent of their lipid-lowering action: the potential molecular mechanisms

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Abstract. – Cerebral ischemia is a major neurological disorder and a leading cause of death and disability in the world. Statins are a widely used group of cholesterol-lowering agents that act by inhibiting the enzyme 3-hydroxy 3-methylglutaryl CoA (HMG CoA) reductase, which catalyses the rate-limiting step of cholesterol biosynthesis. In addition to their cholesterol-lowering properties, however, statins exert a number of so-called 'pleiotropic' actions. Data has emerged largely from thromboembolic animal models of stroke and cultivated cells. It is suggested that statins which have efficacy in preventing the stroke pretreatedly may have a positive effect even given post ischemia possibly through their pleiotropic effects. Mechanisms of the protective actions on cerebral ischemia include the inhibition of inflammatory responses, the improvement of endothelial dysfunction, the regulation of apoptosis proteins, the reduction of oxidative damage and the control of other relative endogenous signal pathways. Attempting to review these properties of statins is an exciting work that will improve our understanding on stroke and thus enable us to better use clinically this type of drugs. The present review summarizes available evidences on the effects and the potential molecular mechanisms of statins on cerebral ischemia.

Key Words:
Statins, Cerebral ischemia, Molecular mechanisms.

Abbreviations

TLRS = toll like receptors
MyD88 = myeloid differentiation factor-88
IRAKs = IL-1 receptor-associated kinases
NF-κB = nuclear transcription factor kappa B
$HMGB_1$ = high mobility group box-1 protein
MCP-1 = monocyte chemoattractant protein 1
BBB = blood brain barrier
ICAM = intracellular adhesion molecules
Akt = protein kinase B
VEGF = vascular endothelial growth factor
ROS = reactive oxygen species
RNS = reactive nitrogen species
GPP = geranylpyrophosphate
FPP = farnesylpyrophosphate
GGPP = geranylgeranylpyrophosphate
OH^- = hydroxide ion
O_2^- = superoxide anion
H_2O_2 = hydrogen peroxide
SODs = superoxide dismutases
MAPKS = mitogen-activated proten kinases
ERKs = extracellular signal-regulated kinases
JNKs = c-Jun N- terminal kinases
MEK = mitogen-activated protein kinase kinase
eNOS = endothelial nitric oxide synthase
nNOS = neuronal nitric oxide synthase
iNOS = inducible nitric oxide synthase
RhoA = Ras homolog gene family member A
Rho GTPase = the small GTPase protein
ET-1 = endothelin-1
ox-LDL = oxidized low density lipoprotein
PAECs = porcine aortic endothelial cells
PARP-1 = poly [ADP-ribose] polymerase 1
EPCs = endothelial progenitor cells

Introduction

According to the American Heart Association, cerebral ischemia is the second leading cause of death and disability among numerous cardiac and cerebrovascular diseases, which ranks only second to coronary heart disease, turning to a severe threaten to human beings[1]. It is well known that the cerebral thrombus, cerebral infarct and systematic hypoperfusion all could cause cerebral ischemic damage due to the limitation of brain blood flow. The deficiency of blood flow results in cellular homeostasis imbalance because of no sufficient oxygen and glucose, then releases a serious of toxin factors to activate certain enzymes or receptors, finally leads to cell death[2]. Therefore, the therapy for cerebral ischemia directs at

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providing enough the cerebral blood flow (CBF) to the areas, reducing the release of toxin factors, suppressing the enzyme/receptor activities, and lowering the extent of cell damage.

Statins, inhibitors of HMG-CoA (3-hydroxy-3-methylglutary coenzyme A) reductase, are cholesterol-lowering drugs widely used because of their high efficacy and safety as well as good tolerance[3]. Therefore, the drugs have received extensive attention since their coming on the scene. Nowadays, evidences from retrospective studies suggest that in addition to risk reduction statins treament may improve stroke outcome via various protective mechanisms. In this article, we summarize recent findings on statins' neuroprotection and potential molecular mechanisms especially on the independent lipid-lowering effects on cerebral ischemia.

Neuroprotection Mediated by Toll-Like Receptors (TLRS)

TLRs are a family of transmembrane receptors that initiate signals in response to diverse pathogen-associated molecular patterns. Thus far, 11 human and 13 mouse TLRs have been identified, mediating the production of endogenous ligands induced by kinds of microorganisms and injuries. They are ubiquitously present in either immune or non-immune cells and their expression are rapidly altered in response to outside stimulations. Until recent years, TLRs have predominantly been examined for their contribution to immune-related disorders[4]. However, cumulative evidences suggest that TLRs not only contribute to pathophysiology, but also play a vital role in facilitating cerebral ischemic conditions.

TLR_4, the first identified mammalian TLR, is implicated in the activation of many signal proteins which result in transcription of gene encoding inflammation-associated molecules and cytokines[5] (Figure 1). Regarding as a portal protein of inflammation and taking part in immune defense responses, the receptor is present in endothelial cells, monocytes, dendritic cells (DCs) and on the surface of myocardial cells. A large number of reports indicate that brain edema, infarct sizes and neural lesions are significantly reduced in TLR_4-deficient (TLR_4KO) mice after occlusion of the middle cerebral artery (MCAO) or ischemia plus reperfusion injury (I/R), showing the vital effect of TLR_4 in cerebral ischemia-induced injury[6-8].

Inhibition of the NF-kB Activity

It has been shown that the innate immune system employs at least three type of membrane receptors on cells, CD14, TLR4 and MD-2, in order to recognize bacterial lipopolysaccharide (LPS)[9-12]. LPS is in close proximity to TLR4 only when it is present as an LPS-CD14 complex and CD14 greatly enhances the formation of LPS–TLR4-MD-2 complexes[13-14] (Figure 1). The concept is that LPS initially binds to CD14 and is then transferred to TLR4. Meanwhile, heat-shock protein HSP70 and HSP90, either on cell surfaces or in cells, could associate with the TLR4-MD2 complex in response to LPS stimulation[15-16] (Figure 1). Moreover, the levels of several classes of HSP were significantly higher in rats after focal cerebral ischemia[17] and also increased in patients with cardiovascular diseases that in healthy control subjects[18].

Activation of TLR_4 recruits an adaptor protein called myeloid differentiation factor-88 (MyD88), which can promote IL-1 receptor-associated kinases (IRAKs) and result in the nuclear translocation of nuclear transcription factor kappa B (NF- B), and then initiate the transcription of genes related with innate immune responses and inflammation (Figure 1). This pathway is called the TLR_4-mediated MyD88-dependent pathway. Studies have shown that the signal pathway was activated after ischemia/reperfusion injury[19]. Research showed NF-κB DNA binding activity and the level of p-I Bα were significantly increased in the control group but lowered in TLR_4KO ones[20]. Simvastatin has been confirmed to suppress the stimulation of NF-kB in a rat model of permanent middle cerebral artery occlusion (pMCAO). The binding activity of NF-kB in animals that treated with simvastatin before pMCAO was significantly decreased compared with the sham-operated rats, indicating that the potential neuroprotection of simvastatin against cerebral ischemia was involved in the inhibition of the activity of NF-kB[21]. Wang et al[22] validated that atorvastatin administration notably and dose-dependently reduced brain edema and infarct size in MCAO-mice and significantly suppressed the up-regulation of high mobility group box-1 protein ($HMGB_1$), $HMGB_1$ receptors (RAGE and TLR_4) and NF-kB at both the protein and the mRNA levels, which may open new perspectives for therapeutic targets of atorvastatin in patients with cerebral ischemia.

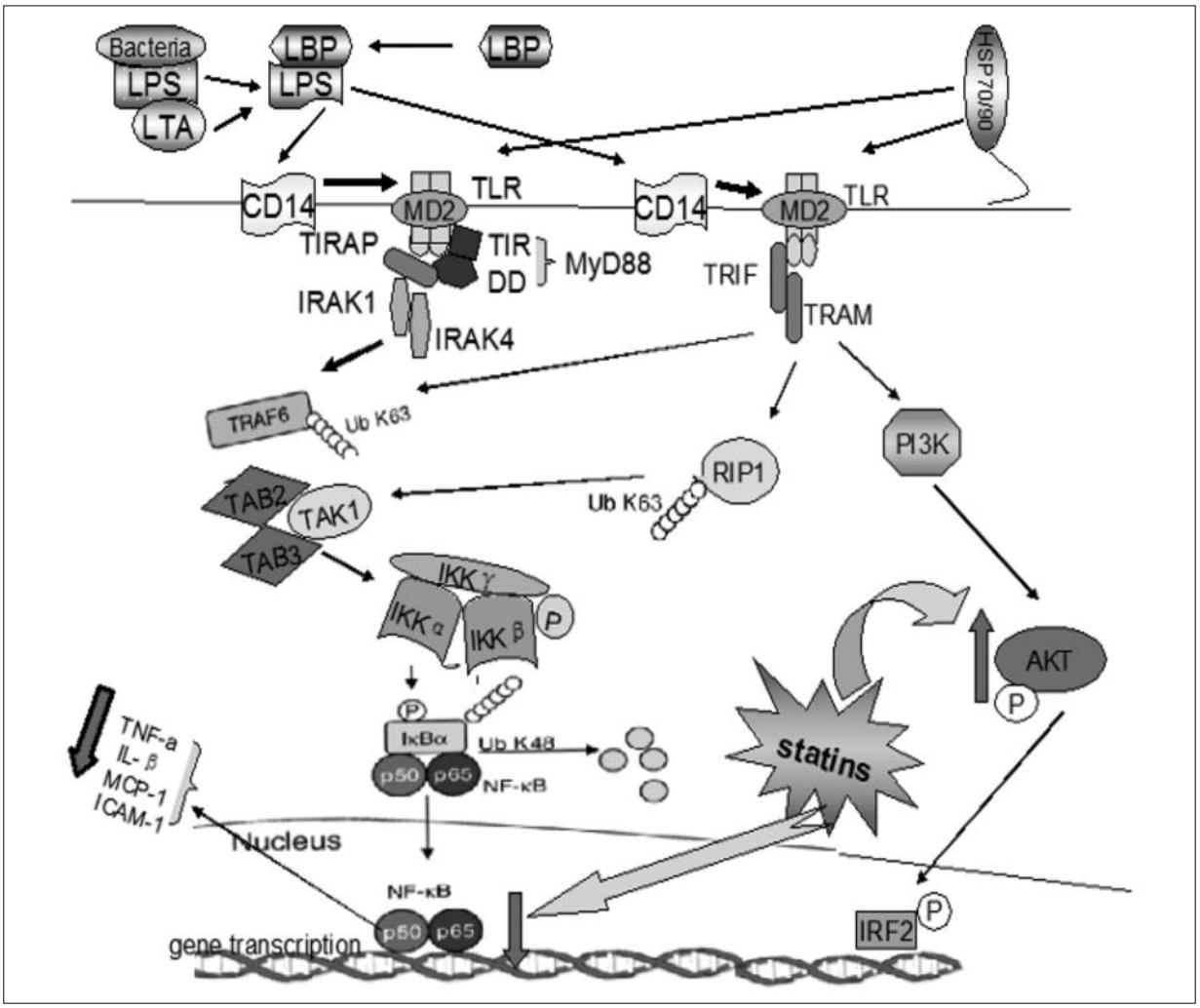

Figure 1. The TLR-mediated MyD88-dependent and independent pathway was activated after ischemia/reperfusion injury, Statins significantly decreased the binding activity of NF-kB p65 and so as to inhibit the release of proinflammatory factors, chemokines and adhesion molecules. They could also upregulate the level of the phosphorylated Akt and show a striking neuroprotection independent on their lipid-lowering effects.

Inhibition of the Release of Proinflammatory factors and Chemokines

Proinflammatory factors such as TNF- , IL-6 and IL-1 , all of which are controlled by NF-kB, play a crucial role in the pathophysiological process of cerebral ischemia. Chemotactic factors (CF), such as monocyte chemoattractant protein 1 (MCP-1), are also important enhancers in post-ischemic inflammation. They have been reported to promote infiltration of leukocyte into ischemic brain areas[23-24]. Their expression could be upregulated by NF-kB. These factors can lead to brain edema and cell infiltration through destroying the blood brain barrier (BBB), and then aggravate the damage of neural function and brain tissues. For the reason that TNF-α and IL-1β in TLR_4KO mice are obviously lower than mice with normal TLR_4, it is possible that the pivotal pathomechanism of TLR_4 in cerebral ischemia-reperfusion is firstly to activate innate immune ligands, then NF-kB then, through, inflammatory cascades release a great deal of cytokines like TNF-α and IL-1β, finally lead to brain injuries[25] (Figure 1). Pravastatin not only a potent neuroprotective compound when applied as a secondary prophylactic drug but also a primary treatment in rats undergoing a temporary MCAO, alleviated the damage of brain tissue in the early phase of ischemia by restraining IL-6 release[26]. Single treatment with atorvastatin also significantly reduced the number of positively stained cells for these inflammation markers as

TNF-α in the ischemic core and penumbra after transient middle cerebral artery occlusion (tMCAO). However, the combination of amlodipine plus atorvastatin showed a synergistic effect for protecting ischemic brain, suggesting that drug combination was a promising clinical therapeutic strategy for ischemic stroke[27].

Inhibition of the Release of Adhesion Molecules

Inflammatory responses mediated by intracellular adhesion molecules (ICAM) such as ICAM-1 are also affected by NF-kB. Reverse transcription polymerase chain reaction (RT-PCR) showed that simvastatin remarkably relieved the damages of the brain and lowered the expression of ICAM-1 mRNA in cortex and hippocampus of neonatal rats when hypoxic-ischemic brain damage (HIBD) occurred, presenting a dramatic positive action[28]. Furthermore, atorvastatin pretreatment could significantly suppress $HMGB_1$-induced vascular endothelial activation in vitro via regulating ICAM-1, E-selectin and TLR4 expression, as well as NF-kB activation, demonstrating that the potential protection of the drug was partly through regulating the TLR_4/NF-kB signal pathway[29]. Mayanagi et al[30] also confirmed that rosuvastatin pretreatment could significantly reduce brain edema, improve blood flow of cerebral cortical and downregulate the level of ICAM mRNA in the lesioned cerebral hemisphere of obese-mice after tMCAO, showing a striking neuroprotection independent on its lipid-lowering effects.

Up-regulation of the Phosphorylated Akt

It was reported that phosphatidylinositol 3-kinase (PI_3K)/Akt signal pathways were involved in the neural protection of ischemic brain damage[31]. Phosphorylated serine/threonine-specific protein kinase (p-Akt), one of the essential physiological regulators in PI_3 pathway, could modulate the function of cells via activating downstream targets in the signal path (Figure 1). Studies have shown that the level of p-Akt in TLR_4KO mice was higher than that in control group after brain I/R; however, that in TLR_2KO mice was significantly lower than that in TLR_4KO mice, showing that there were some correlations among TLR_2, TLR_4 and PI_3K/Akt signal pathway when the cerebral I/R injury occurred. TLR_4 deficiency may enhance the activation of PI_3K/Akt signal in ischemic brain tissues but TLR_2 deficiency may inhibit it[20]. In the immature mice model of hypoxia-ischemia (HI), study confirmed that treatment with simvastatin did enhance Akt phosphorylation in the ipsilateral side of ischemic brain and PI_3K/Akt inhibitor wortmannin could block the phosphorylation and prevent the protective effect of simvastatin, manifesting that the anti-cerebral ischemic effect of the statin was probably connected with the PI_3K/Akt signal pathways[32]. Rosuvastatin, administered intraveniously at a low dose, also significantly reduced lesion sizes after MCAO via increasing the level of p-Akt in blood circulation and the protection was still evident as late as 5 days after brain ischemia[33]. Zhang et al[34] testified that treatment with atorvastatin alone and in combination with tPA significantly increased Akt phosphorylation compared with saline and tPA alone, and the neuroprotection was almost abolished when PI_3K/Akt pathway inhibitor was given. MCAO upregulated early growth response 1 (Egr-1) and vascular endothelial growth factor (VEGF) at mRNA level, while combination of atorvastatin and tPA significantly suppressed that in cerebral endothelial cells, indicating that the combination had further neuroprotective effect on cerebral ischemia by activating PI_3K/Akt pathways as well as suppressing levels of Egr-1 and VEGF.

Neuroprotection Mediated by Vasoactive Cytokin NO and Endothelin

Vascular endothelium forms the inner lining of blood vessel and serves as a physical barrier in the healthy vasculature. In addition, many intrinsic substances, such as oxygen radical nitric oxide (NO), prostacyclin, endothelium-derived hyperpolarizing factor and endothelins, can released from endothelial cells which have secretion ability. Endothelial dysfunction, defined by decreased bioavailability of endothelium-derived nitric oxide synthase (eNOS), is one of the earliest manifestations in cardial and cerebral vascular diseases.

Increase of the Production of Beneficial NO

NO in human body is produced under the catalizing of three enzymes, eNOS, neuronal nitric oxide synthase (nNOS) and the inducible nitric oxide synthase (iNOS). The one produced by eNOS functions as a beneficial signaling molecule in the vascular or other systems and exerts a protective effect when patients suffer from ischemic stroke, partly due to its vasodilative ef-

fects that are helpful to increase cerebral blood flow around ischemic penumbra or damaged regions[35-36]. Statins could increase the initial and beneficial NO production through interfering with eNOS and suppress NO overproduction by downregulating nNOS and iNOS[37]. Moreover, in rodent models of stroke, statins augmented CBF and conferred significant protection partly via mechanisms related to the upregulation of eNOS[38,39].

Inhibition of the Isoprenylation of Rho

Statins due to their HMG-CoA inhibitory activity can block synthesis of a number of isoprenoid intermediates (Figure 2). Isoprenoids include geranylpyrophosphate (GPP), farnesylpyrophosphate (FPP), geranylgeranylpyrophosphate (GGPP), and squalene, which themselves have important biologic functions. FPP and GGPP serve as important lipid attachments for posttranslational modification of heterotrimeric G-proteins and small GTP-binding proteins. Isoprenylation converts small GTPase from a cytosolic (inactive) state to a membrane-bound (active) state (Figure 2). Evidences indicated that direct upregulation of eNOS by statins was at least in part mediated through inhibiting Rho isoprenylation, Rho translocation to cell membrane is inhibited and the downstream activation of Rho kinase (ROCK) is reduced[40-42]. Statins markedly increase Rho gene transcription and chronic statin treatment leads to the accumulation of nonisoprenylated Rho in the cytosol. Cessation of statin treatment

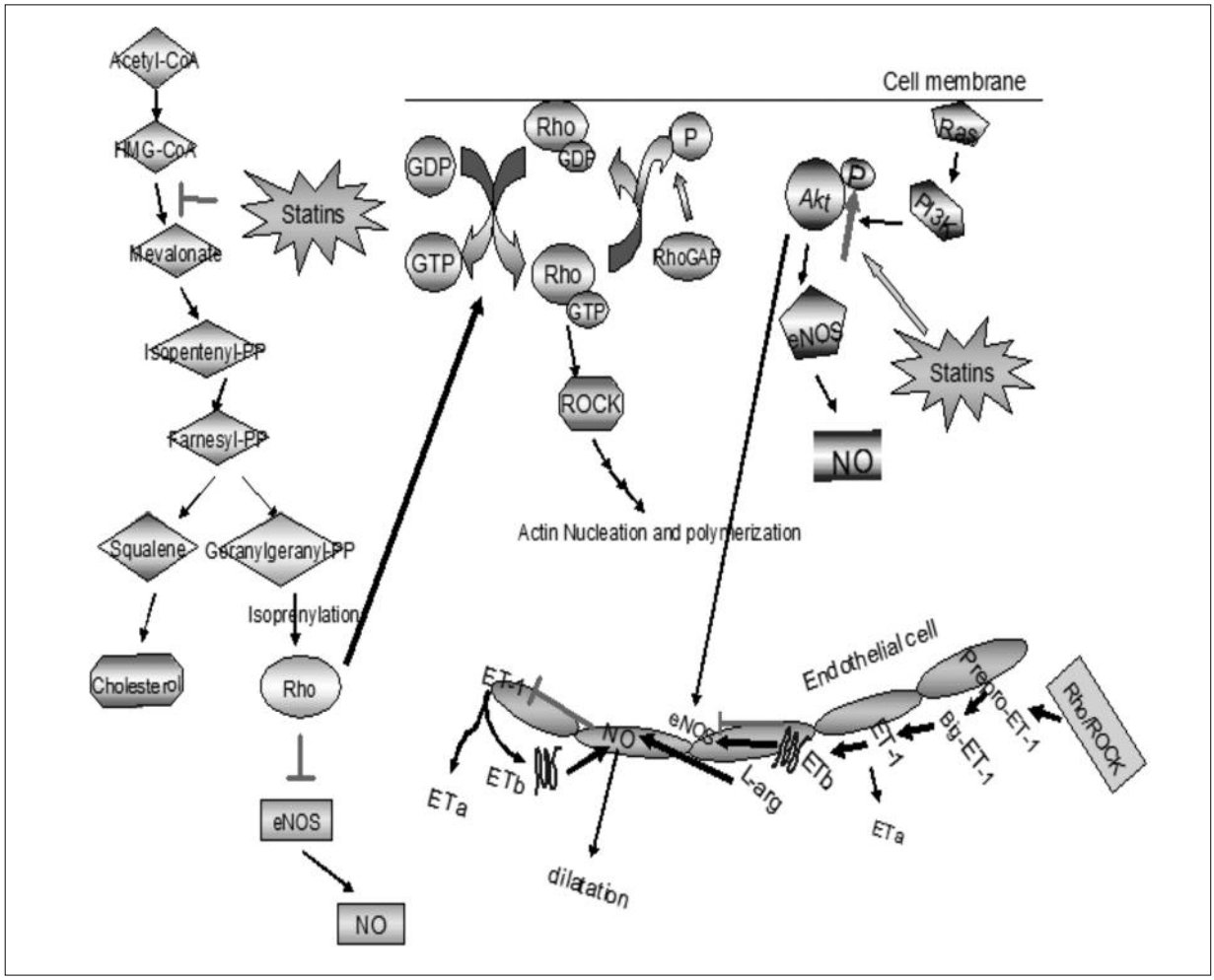

Figure 2. Statins (HMG-CoA reductase inhibitors) inhibit the conversion of HMG-CoA to mevalonate and thereby prevent the formation of isoprenoids and cholesterol. Via inhibition of geranylgeranylation, statins inactivate small G-proteins Rho, then upregulation of endothelial nitric oxide synthase (eNOS) leading to increased NO bioavailability. In addition, statin increases NO production and improves endothelium-dependent vasodilation in a PI-3K and Akt-dependent manner. In the endothelial cell, NO production also might result in the suppression of ET-1 production.

confers overshoot activation of heterotrimeric G-proteins Rho, causing downregulation of endothelial NO production and suppression of NO bioavailability[43,44] (Figure 2).

Activation of Protein Kinase B (Akt)

Moreover, preconditioning-induced eNOS expression via phosphatidylinositol 3 kinase PI-3K pathways after transient forebrain ischemia plays an important role in neuroprotection in the ischemic tolerance[45,46]. Statins acutely increase NO production and improve endothelium-dependent vasodilation in a PI-3K and Akt-dependent manner (Figure 2). The rapid activation of PI3-K and Akt by statins may possibly involve inhibition of Ras homolog gene family member A(RhoA) and ROCK pathway, which is most likely mediated through phosphorylation of eNOS at serine 1177, the major phosphorylation site by Akt[47].

Decrease of the Expression of Endothelin-1

Apart from increasing the beneficial NO production, statins downregulated as well the expression of vasoconstrictive peptide endothelin-1 (ET-1), which was helpful to further improve cerebral blood flow[48]. ET-1, a 21-amino acid component of the peptide-related family, is a major isopeptide synthesized in endothelial cells. It has powerful vasoconstrictive effects compared with other peptides. Moreover, an appropriate balance between NO and ET-1 is struck when the endothelium is in dysfunction. Hernandez-Perera et al[49] found that atorvastatin and simvastatin inhibited pre-proET-1 mRNA expression in a concentration- and time-dependent fashion and lowered immunoreactive level of ET-1. This inhibitory effect was maintained in the presence of oxidized low density lipoprotein (ox-LDL). Mevalonate but not cholesterol reversed the statin-mediated decrease of pre-proET-1 mRNA levels. Expression of eNOS mRNA was dose-dependently reduced by oxidized LDL but not by native LDL. Statins were able to prevent the inhibitory action exerted by oxidized LDL on eNOS at both mRNA and protein levels. Hence, they might influence vascular tone by modulating the expression of endothelial vasoactive factors[49. In addition, studies in porcine aortic endothelial cells suggested that cerivastatin-induced enhancement of eNOS phosphorylation through the PI3-kinase/Akt pathway and the subsequent increase in NO production might result in the suppression of ET-1 production[50] (Figure 2).

Collectively, statins mediate the increase of eNOS expression and activity that upregulates NO production and improves endothelial function. They decrease the ET-1 expression as well. The overall effects on endothelial function are beneficial to improve cerebral blood flow, which at least partially is helpful to explain the neuroprotective effects of statins in ischemic stroke.

Neuroprotection Mediated By Reactive Species

Oxidative stress state is known as much more highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated when organism body is subjected to a variety of noxious stimuli. The state results in a persistent imbalance between the production and the compensation of ROS and then leads to tissue damages[51].

Superoxide is the first ROS generated in the oxygen free radical chain during the early phase of reperfusion and contributes to neuronal injury[52]. Nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase is a principal enzyme for the production of superoxide. Activation of NADPH oxidase leads to an increased generation of the superoxide ion, a ROS that can be converted to a highly reactive hydroxyl ion (OH^-) and a peroxynitrite. There is emerging evidence that this membrane enzyme contributes significantly to ROS generation following cerebral ischemic/reperfusion[53-54]. Raz et al[55] suggested that the small GTPase Rac1 had a critical role in mediating ischemia/reperfusion injury-induced NADPH oxidase activation, ROS generation and oxidative stress in the hippocampal CA1 region of the rat, and, thus, resulted in neuronal degeneration and cognitive dysfunction following cerebral ischemia. Statins could inhibit the activation of Rac1 by preventing the geranylgeranyldependent translocation of Rac1 from the cytosol to the cell membrane[56] (Figure 3). Moreover, atorvastatin could directly suppress NADPH oxidase activity, so as to decrease superoxide level and reduce cerebral infarction[57] (Figure 3).

Evidence showed that statins exerted neuroprotection also via their supression on oxidative stress[58-59]. Massive amounts of ROS are produced and released especially when I/R in neural tissues occurs. The lethal process is accompanied not only by elevated free radicals like su-

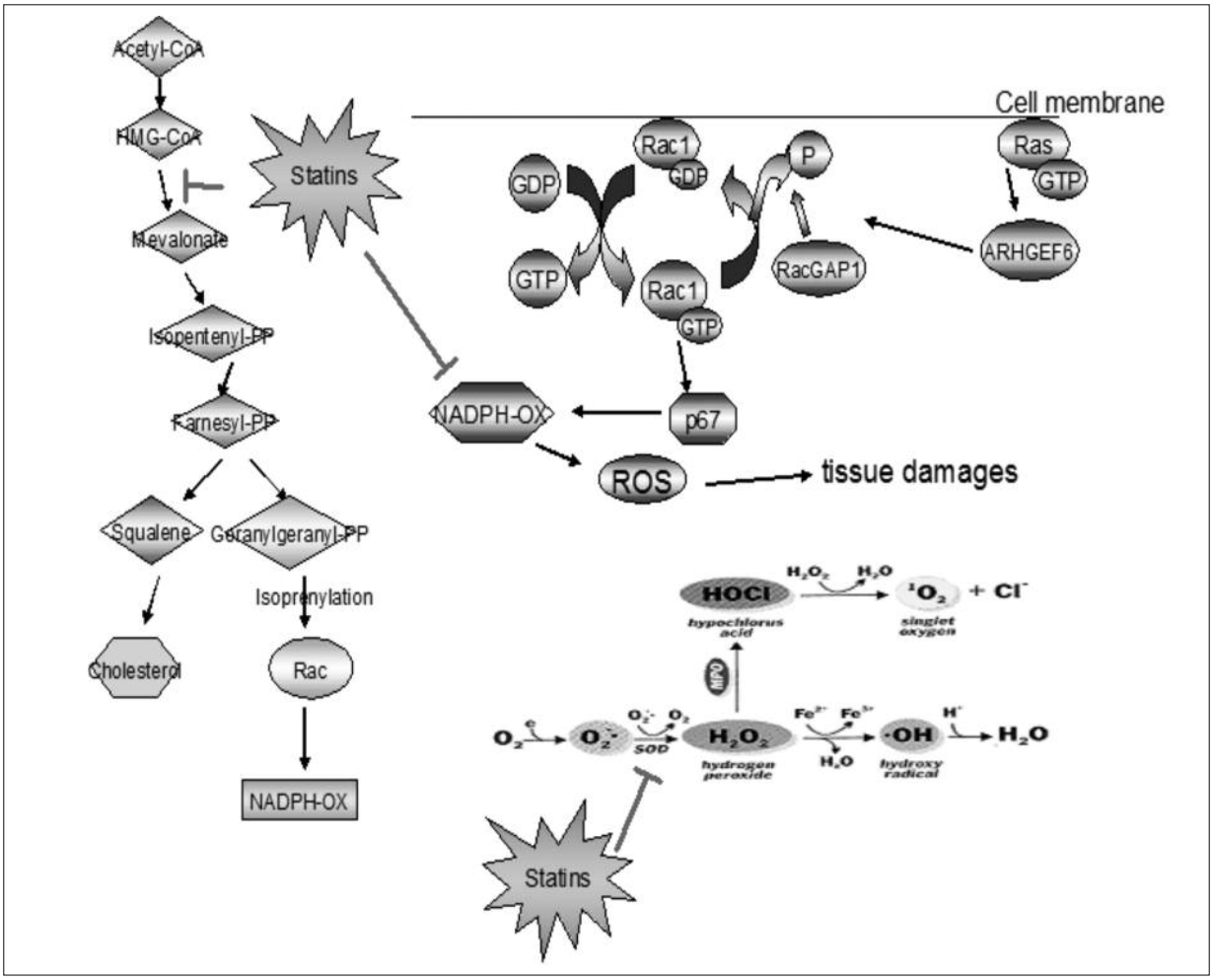

Figure 3. Statins (HMG-CoA reductase inhibitors) inhibit the conversion of HMG-CoA to mevalonate and thereby prevent the formation of isoprenoids and cholesterol. Via inhibition of geranylgeranylation, statins inactivate small G-proteins Rac. then inhibits NAD(P)H-oxidase activity and superoxide production. Also, statins suppresses NADPH oxidase enzymatic activity and SOD directly, exerting significant neuroprotection.

peroxide anion ($O_2^{\bullet-}$), (OH^-) and hydrogen peroxide (H_2O_2) but also by progressive depletion of endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase or antioxidants like glutathione, vitamin C and E[60-61]. ROS may directly damage the main constituents in cells such as lipids, proteins, and nucleic acids and, thus, cause ischemic cell death. It has been reported that pitavastatin treatment preserved SODs immunoreactivity which otherwise became decreased in brains after ischemia[62] (Figure 3). Moreover, simvastatin also showed stronger anti-oxidative properties against ischemic brain damage than other statins presumably because the lipophilic drug was more easily to pass through BBB and, therefore, enter brain parenchyma[58].

Neuroprotection Mediated By Mitogen-Activated Protein Kinases (MAPKS)

MAPKs, serine/threonine protein kinases, play a crucial role in the transduction of extracellular signals in cells and cause a variety of biological responses, like cell proliferation, differentiation, transformation and apoptosis. They include various signaling proteins, such as extracellular signal-regulated kinases (ERKs), c-Jun N- terminal kinases (JNKs) and p38 MAPKs, which are related to neuronal injuries and diseases.

Regulation of the Level of Phospho-ERKs

ERKs, proline-driven serine/threonine kinases, can phosphorylate serine/threonine which are ad-

jacented with proline and accept the upstream cascade signals to enter the nucleus after mitogen is stimulated. Both ERK1 and ERK2 exert important effects on ischemic neuron injury when they are activated by the MAPK/ERK1/2 pathway. They are phosphorylated and activated through a three-tiered mitogen-activated protein kinase kinase (MEK) mode by the signal transduction of cell surface receptors stimulated by growth factors or cytokines (Figure 4). The p-ERK elevation is associated with both beneficial and detrimental neurological effects after stroke and statins contribute to ischemic damage by regulating the level of phospho-ERKs (Figure 4).

Levels of phospho-ERK1/2 are usually increased after cerebral ischemia/reperfusion. It may actually exacerbate neurological damages, increase oxidative stress-related death, and promote inflammations. Sironi et al[21] demonstrated that the protection of simvastatin from cerebral ischemia was connected with MAPKs pathways. The level of phospho-ERK1/2 was elevated in ischemic hemispheres and reached the highest expression 2 h after injury. Administration of simvastatin before MCAO could reduce the level. U0126, a selective MEK pathway inhibitor, given after MCAO, markedly decreased the MCAO-induced ERK1/2 phosphorylation and reduced infarct sizes, authenticating that the potential protective mechanism of simvastatin was dependent on its suppressing effect on MCAO-induced ERK1/2 phosphorylation.

Conversely, some other conflicting data were also reported about the role of ERKs in cerebral ischemia. Expression of p-ERKs is various in different regions of ischemic brain. It was found in the penumbra more than in the ischemic core. This activation pattern has led many to believe

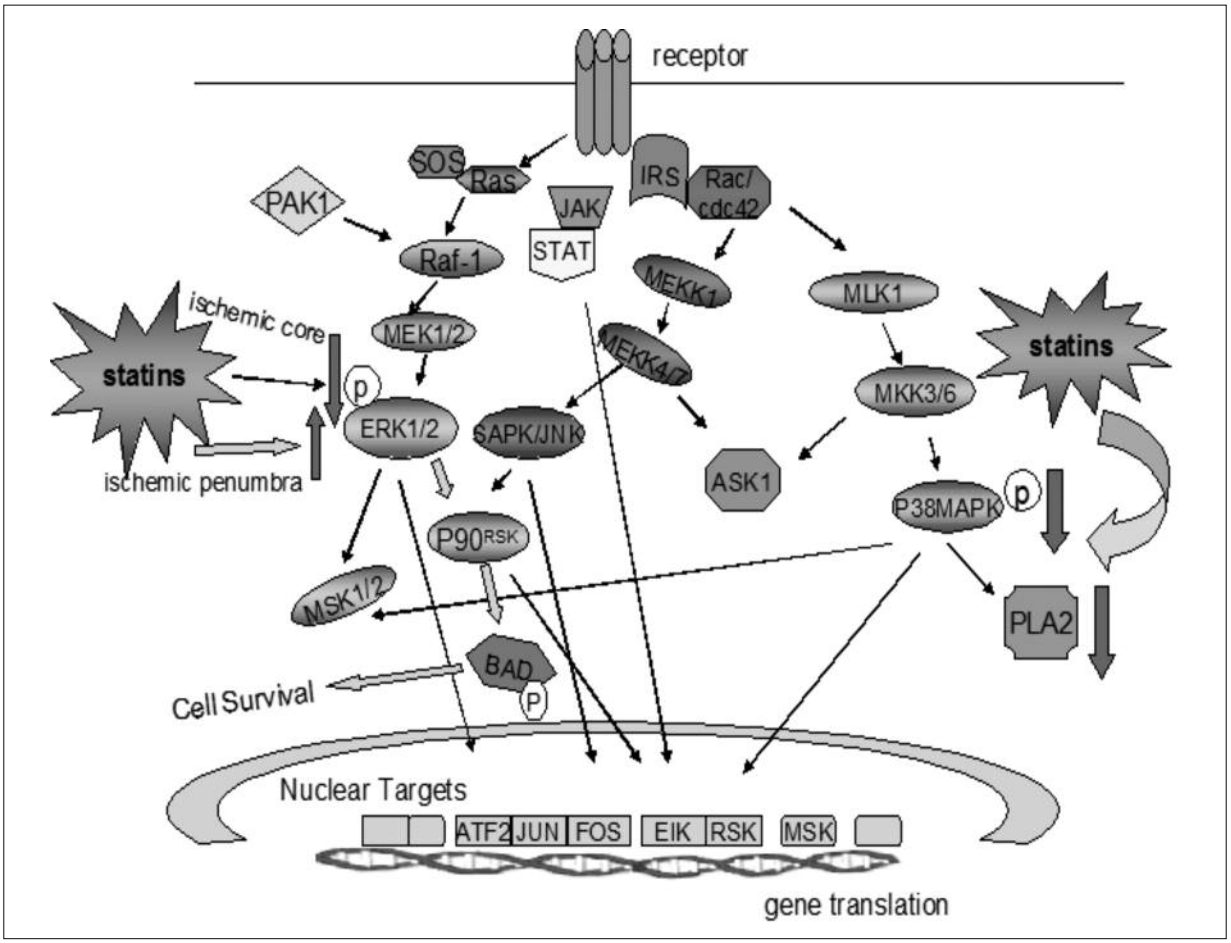

Figure 4. MAPKs, including various signal proteins, such as extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs) and p38 MAPKs, play a crucial role in the transduction of extracellular signals and cause a variety of biological responses. Statins could regulate the levels of phospho-ERKs and decrease the expression of p38 MAPKs, excerting an observious neuroprotection on cerebral ischemia.

that the elevated ERKs-phosphorylation played a role in the cell survival. Simvastatin lactone, which was a prodrug and could easily be across BBB, provided a high-grade protection from focal brain ischemia/reperfusion injury. It activated the survival kinases ERKs in ischemic hemispheres, enhanced the endogenous anti-oxidant capacity of brain tissues and increased the neural survival factors in ischemic regions and the levels of phospho-ERK1/2 induced by cerebral ischemia. The results indicated that the possible mechanism of the drug might be associated with endogenous protection pathways such as the activation of ERKs and the increase of anti-oxidant activity[63].

Decrease of the Expression of p38 MAPKs

p38 MAPKs, which participate in inflammatory responses and cell apoptosis, are stress-activated proteins. Western blot analysis indicated that the activation and phosphorylation of p38 MAPKs were markedly increased after focal cerebral ischemia and reperfusion, and the suppression of the activation could effectively attenuate BBB injury and brain edema and showed a certain extent of neuroprotective effects[64].

Atorvastatin notely reduced brain edema and infarct sizes and the overexpression of 12/15-LOX, p38 MAPKs, phospho-p38 MAPKs and cPLA2, declearing a new target. That atorvastatin protected from cerebral ischemic injury might be in inhibiting 12/15-LOX/p38MAPKs/cPLA2 pathways of arachidonic acid metabolism. Moreover, p38 MAPKs, phospho-p38 MAPKs and cPLA2 were upregulated by atorvastatin 3 h after MCAO and they went to a high level at 24 h and to the climax at 48 h, indicating that the drug impacted the pathologic process of cerebral ischemia mainly at the early stage[65], Céspedes-Rubio et al[66] also made an evaluation on the effect of atorvastatin. Results showed that treatment of the drug drastically decreased the level of p38 MAPKs in cerebral cortex and hippocampus 6h after I/R, and reduced the infarct volume and neural injury, displaying availably neuroprotection on focal cerebral ischemia.

Neuroprotection Mediated by Apoptosis-Related Proteins Caspases and BCL-2

A key event that causes brain ischemia/reperfusion injury is the apoptosis of neurons. The cerebral damaged sizes depend on the extent of apoptosis neurons. After cerebral I/R injury, the pathological changes in the center of ischemia are mostly necrosis while in the peripheral area mainly apoptosis. Apoptosis is a gene controlled cascade process, where the protein family caspases and Bcl-2 are involved in[67] (Figure 5).

Reduction of the Caspase-3 Activation

Caspases are key homologous homocysteine proteases regulating apoptosis (Figure 5). Homologating with the nematode death gene ced-3, they can initiate and implement the apoptosis of mammal cells and cleavage specific target proteins followed aspartic acid-residue. So far, 14 caspases have been found, among which caspase-2, -3, -6, -7, -8, -9 and -10 are directly related with cell apoptosis. Caspase-3 is considered as the most important protease in caspase-related cascade of various apoptotic signal pathways. If an apoptosis process is made all events finally must be through the activation of caspase-3 (Figure 5).

One of the targets for caspase-3 to cleavage is poly [ADP-ribose polymerase 1 (PARP-1) which has an 89-kDa C-terminal site containing catalytic domain and a 24-kDa one binding with DNA ending. Thereby the energy-dependent apoptotic process is able to be successfully completed. Carloni et al[68] corroborated that expression of intact PARP-1 (116-kDa) could be detected in brains of all animals, but lower quantity of its 24-kDa fragment was only appeared in the ischemic cortex of the damaged side. The expression of 116-kDa in the simvastatin group was significant higher but the 24-kDa fragment lower than those in other groups. The expression of intact PARP-1 in ischemic regions of simvastatin-treated animals indicated that the cleavage of the protein in the brain reduced. Simvastatin significantly suppressed the increase of caspase-3 expression and activity observed 24 h after ischemic insult contemporarily, affirming that the drug reduced the apoptotic signal transduction induced by hypoxic-ischemia in neonatal rats through reducing the cleavage of PARP-1 and lowering the caspase-3 activation.

Endothelial progenitor cells (EPCs), the progenitor of endothelial cells, also known as vascular cells or angioblasts, can mobilize from bone marrow to peripheral bloodstream and participate in repairing the injured vessels when they are stimulated by physiological or pathological events such as ischemia. High concentration of homocysteine (Hcy) is a risk factor for cardiovascular diseases and stroke, which can induce the

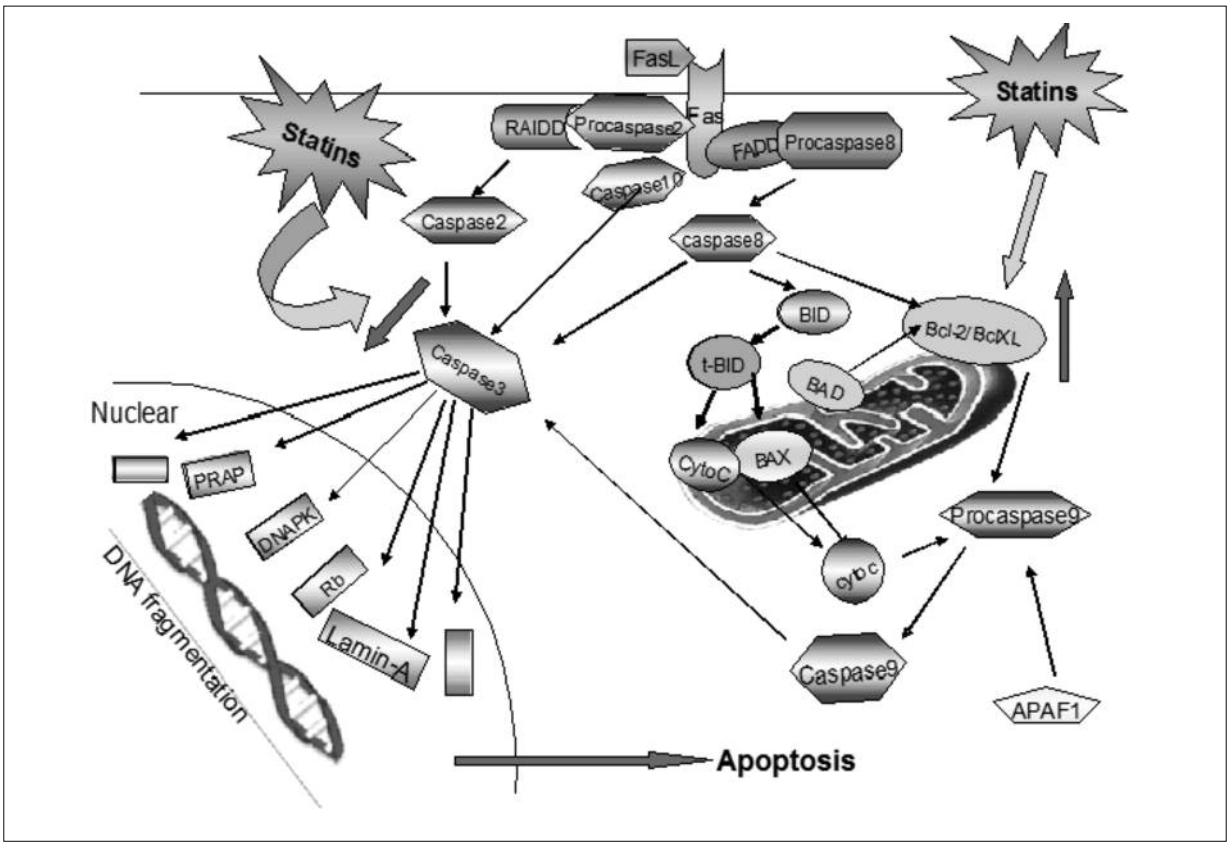

Figure 5. Apoptosis is a gene controlled cascade process. The pathological changes in the center of ischemia are mostly necrosis while in the peripheral area mainly apoptosis after cerebral I/R injury. Statins present a dramatic neuroprotective effect through reducing the caspase-3 activation and increasing the Bcl-2 expression.

vascular dysfunction and even lead to ischemia. Alam et al[69] using multiple regression analysis obtained that the serum Hcy in ischemic patients had a certain correlation with EPCs: every 10 mmol/L increased in fasting homocysteine, colony counts of EPCs decreased on the average by 7.4. *In vitro*, homocysteine dose-dependently increased the caspase-3 in EPCs, however, treatment with Z-VAD-FMK, a global caspases inhibitor, decreased the caspase-3 activation.

A study found that atorvastatin could effectively inhibit Hcy-induced endothelial progenitor cell dysfunction and apoptosis[70]. Hcy does-dependently impaired the proliferation, migration and *in vitro* vasculogenesis capacity of EPCs and induced cell apoptosis. Atorvastatin pretreatment could significantly attenuate the detrimental effects of Hcy, improve the function of EPCs, inhibit the apoptosis of EPCs and markedly lower Hcy-induced caspase-3 activity in a dose-dependent manner. It showed that the drug inhibited the apoptosis of EPCs to a certain extent by down-regulating the caspase-3 activity.

Increase of the Bcl-2 Expression

Apoptosis controlled by both intracellular and extracellular factors is an active way of cells to death in physiological and pathological conditions. Cysteine protease caspases are important elements to initiate apoptosis. Bcl-2, Bax and some other the family members can involve in regulating the caspases pathway (Figure 5) and so indirectly affect the occurrence of apoptosis in diseases.

The expressing product of Bcl-2 gene is one kind of 26 kD membrane protein Bcl-2, which can inhibit the activation of downstream caspase-3 by blocking the release of cytochrome C so as to suppress the occurrence of apoptosis. Meanwhile, it is the substrate of caspase-3 and can be hydrolyzed by the caspase. Bcl-2, an inhibitor of apoptosis in neuronal and glial cells, is highly expressed in the embryonic and developing mammalian brain. Events have well documented that altering the levels of anti-apoptotic molecules, such as Bcl-2, could modulate apoptosis followed brain damage. Zhang et al[71] has

provided that Bcl-2 treatment reduced the infarct volume and the number of degenerating cells, increased the level of neural progenitor cells as well as newborn immature and mature neurons, downregulated the apoptosis rate of newborn striatal neurons in rat brains followed a transient occlusion of the middle cerebral artery, hinting that Bcl-2 could be beneficial to increase neurogenesis and survival of newborn neurons in adult brain, which may be a strategy to help for the repair of brain after cerebral ischemia. Western blot analysis showed that[72] treatment of simvastatin for 21 days significantly increased anti-apoptotic Bcl-2 protein levels in brains of the drug-treated animals as compared with the control. Protein levels of pro-apoptotic Bax, the major opponent of Bcl-2, were significantly reduced. The Bax/Bcl-2 ratio, a crucial parameter for regulating apoptosis, was also decreased in brain tissues of simvastatin-treated animals. Collectively, the neuroprotective effects of simvastatin were mediated in part by up-regulation of Bcl-2 and down-regulation of Bax protein levels. *In vitro* studies[73,74] also showed that mRNA and protein levels of Bcl-2 are significantly increased in rat primary cortical neurons and human ganglion cells pretreated with simvastatin, indicating that the inhibition of simvastatin on neuronal apoptosis is concerned with promoting cell survival gene bcl-2 expression.

Conclusions

Statins have preferable neuroprotective and preventive effects both *in vitro* and *in vivo* via multiple mechanisms but not simply via cholesterol-lowering. Moreover, systematic review and meta-analysis of the efficacy of statins by Baryan et al[75] discovered that the neuroprotection of statins still might have some limitations. Therefore, to enlarge researches on the effects and mechanisms of statins alone or combination of the drugs with other anti-ischemic therapies can deepen the understanding of the etiology of ischemic cerebrovascular diseases and provide a theoretical basis for the development of new anti-cerebral ischemic drugs.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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