The Synergistic Neuroprotective Effects of Combined Rosuvastatin and Resveratrol Pretreatment against Cerebral Ischemia/Reperfusion Injury

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Background: It is well accepted that both rosuvastatin and resveratrol exert neuroprotective effects on cerebral ischemia/reperfusion injury through some common pathways. Resveratrol has also been demonstrated to protect against cerebral ischemia/reperfusion injury through enhancing autophagy. Thus, we hypothesized that combined rosuvastatin and resveratrol pretreatment had synergistic effects on cerebral ischemia/reperfusion injury. *Materials and Methods:* Adult male Sprague Dawley rats receiving middle cerebral artery occlusion surgery as animal model of cerebral ischemia/reperfusion injury were randomly assigned to 4 groups: control, resveratrol alone pretreatment, rosuvastatin alone pretreatment, and combined rosuvastatin and resveratrol pretreatment. Rosuvastatin (10 mg/kg) or resveratrol (50 mg/kg) was administrated once a day for 7 days before cerebral ischemia onset. *Results:* We found that combined rosuvastatin and resveratrol pretreatment not only significantly decreased the neurologic defective score, cerebral infarct volume, the levels of caspase-3, and Interleukin-1β (IL-1β) but also significantly increased the ratios of Bcl-2/Bax and LC3II/LC3I, as well as the level of Becline-1, compared with resveratrol alone or rosuvastatin alone pretreatment group. Rosuvastatin alone pretreatment significantly increased the ratio of LC3II/LC3I and the level of Beclin-1. However, there were no significant differences in the neurologic defective score, cerebral infarct volume, the levels of caspase-3, IL-1β, and Beclin-1, and the ratios of Bcl-2/Bax and LC3II/LC3I between resveratrol pretreatment group and rosuvastatin pretreatment group. *Conclusions:* Synergistically enhanced antiapoptosis, anti-inflammation, and autophagy activation might be responsible for the synergistic neuroprotective effects of combining rosuvastatin with resveratrol on cerebral ischemia/reperfusion injury. **Key Words:** Cerebral ischemia/reperfusion injury—antiapoptosis—anti-inflammation—autophagy activation.

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Introduction

Ischemic stroke is one of the leading causes of mortality and morbidity worldwide, which lacks effective treatments. The pathophysiological mechanisms of ischemic stroke, especially cerebral ischemia/reperfusion (I/R) injury, are complex, including energy metabolism impairment, oxidative stress, glutamate or neurotoxin release, calcium overload, inflammation, apoptosis, and autophagy.[1] In particular, reperfusion injury caused by restoring the cerebral blood triggered various deleterious cascades of ischemic stroke.[2] Although tissue plasminogen activator is still considered to be the only effective treatment, narrow treatment window (4.5 hours) and relatively high risk of intracerebral hemorrhage limited the clinical application of tissue plasminogen activator.[3] Thus, neuroprotective agents are still considered to be more attractive and effective candidate for treating ischemic stroke.

To date, statins are still considered to be the most extensively investigated and promising neuroprotective agents, although statins are not recommended in stroke guidelines as an agent with neuroprotective actions to improve outcome in acute stroke.[4] There is no controversy that statins exert neuroprotective effect on the animal models of cerebral I/R injury[5] via different kinds of mechanisms, including inhibition of inflammation, immunomodulation,[6] antioxidative effects, and induction of beneficial NO,[7] although there is still discrepancy about the effect of statin use on the clinical outcome of acute ischemic stroke.[8,9] Stain intensity and adherence partly ascribe to the controversy. There might be other possible mechanisms, which contribute to the controversy because of the complex pathophysiological process of cerebral I/R injury. Now, more and more evidence indicated that the inflammatory cascade and autophagy mainly contributed to the cerebral I/R injury.[10] It has also been shown that modulating the process of autophagy could improve the outcome of animal models of cerebral I/R injury.[11-13] In addition, the level of autophagy in cerebral spinal fluid is associated with the outcome of ischemic stroke.[14] This finding indicates that the neuroprotective agents targeting both the inflammation cascade and the autophagy might be effective therapeutic strategies for treating cerebral I/R injury. Statins have been confirmed to protect against cerebral I/R injury via inhibition of inflammation. However, to date, there are no lines of evidence to clarify whether statin use has an effect on the autophagy caused by cerebral I/R injury, although statins exert cytoprotective effects by modifying autophagy pathway in several diseases, including Parkinson's disease, the metastasis of cancer,[15] ischemic and nonischemic myocardium,[16] spinal cord injury,[17] and asthma.[18] These findings suggest that agents targeting the autophagy pathways might enhance the neuroprotective effects of statins on the cerebral ischemia I/R injury.

It is well accepted that resveratrol (RES), a natural polyphenol richly found in red wine, has the capacity to protect rats against cerebral ischemia/reperfusion injury.[19,20] Such neuroprotection of RES involved several different mechanisms, which included anti-inflammation, antioxidant, immunomodulation,[19] and antiapoptosis.[20] There are some common pathways between statins and RES, which contribute to the neuroprotective effects on cerebral I/R injury. In addition, RES is considered a natural autophagy regulator.[21] More importantly, it has been demonstrated that RES alleviated cerebral I/R injury in rats through upregulating the level of autophagy.[2] Now, rosuvastatin (ROS) is one of the extensively recommended and investigated statins for primary and secondary prevention of ischemic stroke.[22] In addition, ROS was chosen over other types of statin because of its efficacy, low cytotoxicity, and pleiotropic effects.[23] Thus, we speculate that co-administration of RES and ROS might have synergistic neuroprotective effects on the cerebral ischemia I/R injury.

Thus, in the current study, we used middle cerebral artery occlusion (MCAO) for 90 minutes in rats as animal model of cerebral I/R injury. We tried to investigate whether combined RES and ROS pretreatment had the synergistic neuroprotective effects on cerebral I/R injury in rats. In addition, we also tried to uncover the possible mechanisms in vivo.

Materials and Methods

Animals

Adult male Sprague Dawley rats (n = 60), weighing 200-220 g, were purchased from Beijing Hua Fukang Biotechnology Co. Ltd. (Beijing, China) (license No. SCXK (Jing) 2014-0004). The rats were kept under standard laboratory conditions, maintained in temperature- and humidity- controlled rooms on a 12-hour/12-hour light/dark cycle, and had free access to food and water. All animal protocols and procedures were reviewed and approved by the guidelines of the Ethics Committee on Animal Experiments of Shandong University.

Drug Preparation and Treatment Schedule

Purified resveratrol (RES) was purchased from Sigma-Aldrich (St. Louis, MO). One hundred milligrams of RES was freshly dissolved in 4.5 mL of 1.5% dimethyl sulfoxide (Sigma-Aldrich) and 5.5 mL of .9% saline solution (Baite, Shanghai, China). Fifty milligrams of rosuvastatin (ROS, Crestor, IPR Pharmaceuticals Incorporated, Puerto Rico) was freshly dissolved in 10 mL of .9% saline solution (Baite). RES was administrated intraperitoneally (i.p.) at a dose of 50 mg/kg. ROS was administrated at a dose of 10 mg/kg through intragastric gavage.