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Research Report

Geranylgeranylacetone protects against cerebral ischemia and reperfusion injury: HSP90 and eNOS phosphorylation involved

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ABSTRACT

Cerebral ischemia and reperfusion (I/R) can trigger a cytotoxic cascade with overflow of reactive oxygen species, paradoxically causing neurological dysfunction, redox imbalance, inflammation and apoptosis. The present study aims to investigate the effect of geranylgeranylacetone(GGA) on cerebral I/R injury and the underlying mechanism. The results demonstrated that cerebral I/R increased the neurological function abnormality, brain edema, inflammation and oxidative injury in rats as well as the cognitive impairment, which was significantly reversed by GGA in a dose-dependent manner. GGA also suppressed the cell injury and apoptosis caused by cerebral I/R. Moreover, the protective effect of GGA was found to involve heat shock protein 90 (HSP90) and phosphorylated endothelial nitric oxide synthase (eNOS) expression and activity. Both the HSP90 and eNOS inhibitor abolished the effect of GGA. The data showed that GGA could protect rats against cerebral I/R injury, which may be related to the induction of HSP90 and activation of eNOS.

1. Introduction

Stroke, a leading cause of human mortality, causes almost 5 million deaths in the modern world each year. As the brain needs a quarter of the cardiac output, any deficiency in cerebral blood flow may lead to cerebral ischemia and neurological dysfunctions(Deb et al., 2010). Adequate evidence have shown that a timely blood flow restoration is necessary to treat cerebral ischemia. However, it can also induce reperfusion injury by triggering a cytotoxic cascade with overflow of reactive oxygen species (ROS), paradoxically causing neurological dysfunction, redox imbalance, inflammation and apoptosis(Ozbal et al., 2008; Yousuf et al., 2009), which is called cerebral ischemia and reperfusion (I/R) injury. With the rise of the burden of stroke, it is desperately needed to protect the brain against cerebral I/R injury-induced complications. However, there is no effective

Abbreviations: GGA, geranylgeranylacetone; I/R, ischemia and reperfusion; HSP90, heat shock protein 90; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; AUC, area under curve; MDA, malonaldehyde; TNF-α, tumor necrosis factor-α

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treatment for cerebral I/R injury as yet, although many studies has explored potential therapeutic strategies (Ozbal et al., 2008; Jia et al., 2014; Jung et al., 2006; Liu et al., 2012).

Geranylgeranylacetone (GGA) is an acyclic polyisoprenoid used as an oral anti-ulcer medication. Recently many studies have found out that, by inducing heat shock proteins(HSPs), GGA is protective against various I/R injury, including myocardial I/R injury(Yamanaka et al., 2003), acute kidney I/R injury (Kim et al., 2014), liver ischemia–reperfusion injury (Fan et al., 2005; Yamagami et al., 2000). The role of HSP70 was intensely investigated in these studies, but the role of HSP90, another HSP induced by GGA, was seldom studied. In addition, Yamagami et al. (2005) studied the neuroprotective effect of GGA in permanent focal cerebral ischemia, but its effect on cerebral I/R injury was not examined or reported yet.

Nitric oxide synthase (NOS) includes three different isoforms, including the constitutive neuronal NOS(nNOS), endothelial NOS (eNOS) and the inducible NOS (iNOS). It is accepted that eNOS is protective against cerebral ischemic injury by the following mechanism: NO produced by eNOS can (1) regulate cerebral blood flow; (2) mediate the vascular response to the oxidative stress; and (3) inhibit platelet aggregation, platelet and polymorphonuclear neutrophil adhesion to the vascular endothelium (Liu et al., 2012).

Many studies have reported the interaction of HSP90 and eNOS in various disease prevention (Ramirez-Sanchez et al., 2012; Vladic et al., 2011; Simet et al., 2013). It was reported that Hsp90 inhibitor was able to inhibit the eNOS and Hsp90 interaction and decrease the level of eNOS (Prangsaengtong et al., 2011). Hence, to explore the protective effect of GGA against cerebral I/R injury and the underlying mechanism, we observed the protective effects of GGA on cerebral I/R injury, demonstrated by neurological deficit scores, brain water content, tumor necrosis factor-α (TNF-α) and malonaldehyde (MDA) levels, then examined the expression and interaction between HSP90 and eNOS and their roles in the GGA-induced cerebral protection.

2. Results

2.1. Neurological deficit scores, brain water content, MDA and TNF-α levels in the brain

As shown in Table1, rats exposed to cerebral I/R exhibited significant increase in the neurological deficit scores, brain water content, MDA and TNF-α levels in the brain compared to Sham group ($P<0.05$). The neurological deficit score in the cerebral I/R group (5.58±0.66) was approximately 10 times higher than the Sham group (0.43±0.05). 50 mg/kg GGA decreased the neurological deficit score to 3.34±0.32 and the 200 mg/kg GGA decreased it to 2.18±0.36 ($P<0.05$ compared to 50 mg/kg GGA). The TNF-α in the 200 mg/kg GGA group was also significantly different from the 50 mg/kg GGA, indicating that these parameters were decreased by GGA in a dose-dependent manner ($P<0.05$ compared to cerebral I/R).

2.2. Time course of swimming time and the AUC changes

The time course of the swimming time was shown in Fig. 1A. Swimming time in groups received cerebral I/R injury increased to the highest level at one week after cerebral I/R, then gradually decreased over time. However, the increase of swimming time in cerebral I/R group was significantly bigger than that in the cerebral I/R+GGA group. As shown in Fig. 1B, the AUCs of the swimming time of both the cerebral I/R group and cerebral I/R+GGA group were higher than that in the Sham group ($P<0.05$). Treatment with GGA significantly decreased the AUC as compared to that of the Cerebral I/R group ($P<0.05$).

2.3. Changes in Nissl-positive and apoptotic cell counts

Fig. 2 compared the changes in Nissl-positive (Fig. 2A) and apoptotic cell (Fig. 2B) counts in each group. The Nissl staining was performed in the brain cortex tissue in the sections 1.50 mm anterior to the bregma and 3.5 mm posterior to the bregma. The number of Nissl-positive cells in cerebral I/R group was significantly lower than that in the Sham group ($P<0.05$), while the administration of 200 mg/kg GGA dramatically increase the Nissl-positive cells compared to cerebral I/R group ($P<0.05$). In Fig. 2B, the cerebral I/R greatly increased the TUNEL-positive cells in the hippocampus area in rat brain, which is vulnerable to cerebral I/R injury ($P<0.05$). The GGA administration partly reduced the number of TUNEL-positive cells ($P<0.05$).

2.4. HSP90 and phosphorylated eNOS expression and eNOS activity

As shown in Fig. 3A, the cerebral I/R injury increased the HSP90 ($P<0.05$), which was further enhanced by GGA ($P<0.05$). The HSP90 inhibitor 17-AAG significantly inhibited the HSP90 expression ($P<0.05$), while the eNOS inhibitor L-NIO had no such ability. As demonstrated in Fig. 3B, the phosphorylated eNOS

Table1 – Neurological deficit scores, brain water content, MDA and TNF-α levels in the brain. Sham: rats received gum Arabic before a sham cerebral I/R surgery; Cerebral I/R: rats received gum Arabic before a real cerebral I/R surgery; 50 mg/kg GGA, 100 mg/kg GGA and 200 mg/kg GGA: rats received GGA at a dose of 50 mg/kg, 100 mg/kg and 200 mg/kg before a cerebral I/R surgery. TNF-α: tumor necrosis factor-α; MDA: malonaldehyde. Values are the Mean±SEM.

	Sham	Cerebral I/R	50 mg/kg GGA	100 mg/kg GGA	200 mg/kg GGA
Neurological deficit score	0.43±0.05	5.58±0.66*	3.34±0.32&	3.06±0.57&	2.18±0.36&
Brain water content (%)	68.56±2.48%	86.85±3.21%*	72.43±2.33%&	69.42±2.15%&	68.11±1.91%&
MDA (μmol/mg protein)	1.54±0.11	4.36±0.24*	3.34±0.36&	3.15±0.47&	2. 59±0.44&
TNF-α (pg/mg)	2.67±0.12	8.77±0.34*	6.45±0.42&	4.46±0.61&	4.17±0.55&

* $P<0.05$ compared to Sham.
& $P<0.05$ compared to Cerebral I/R. $N=10$ per group.