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Effects of Panax notoginseng ginsenoside Rb1 on abnormal hippocampal microenvironment in rats

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ABSTRACT

Cerebral ischemia damages central neurons, and abnormal microenvironment in ischemic condition is the key factor to the damages. The increase of local concentration of glutamic acid, the overload of Ca^{2+}, and the mitochondrial stress caused by release of cytochrome C are important factors of abnormal microenvironment in cerebral ischemia. In this study ginsenoside Rb1, a compound from Panax Notoginseng, was used to intervene abnormal environment of neurons in the hippocampal CA1 region in two animal models (microperfusion model and photothrombosis model). Results: Compared with the vehicle in the sham group, ginsenoside had following effects. a) ginsenoside Rb1 increased the regional cerebral blood flow (rCBF) and the stability of neuronal ultrastructure in in the hippocampal CA1 region and improved the adaptability of neurons in two models. b) ginsenoside Rb1 improved the expression level of glial glutamate transporter1 (GLT-1) and reversed the uptake of glutamate (Glu) after ischemia, and as a result thereby decreased the excitability of Glu and the expression level of GLT-1 was proportional to the dose of ginsenoside Rb1 and similar to that of Nimodipine. c) ginsenoside Rb1 inhibited the expression level of NMDAR and the overload of Ca^{2+}, thereby reducing neuronal damages. Meanwhile, the expression level of NMDAR was inversely proportional to the dose of ginsenoside Rb1, which was similar to that of Nimodipine. d) ginsenoside Rb1 decreased the release of cytochrome C (Cyt-C) and reduced the damages caused by neuronal mitochondrial stress. Meanwhile, the release of Cyt-C was inversely proportional to the dose of ginsenoside Rb1, which was similar to that of Nimodipine. Ginsenoside Rb1 may be as an effective drug for neuroprotection and improve cerebral blood flow after acute ischemia and prevent the secondary brain damage induced by stroke.

1. Introduction

Stroke is a disease with high incidence, high disability and high mortality. Ischemic stroke occupies 75–85% of all stroke patients. Ischemic stroke is also known as cerebral infarction, which always occurs with abnormal microenvironment of the brain and causes damage of neurons (Aarts et al., 2002). When cerebral thrombosis forms, the interaction between different factors in neuronal microenvironment amplifies the ischemic insult, and then forms the abnormal microenvironment. The formation of abnormal microenvironment promotes secondary damage of neurons. The intracellular Ca^{2+} overload and increase of glutamate (Glu) are the key factors composing of the abnormal microenvironment (Choi and Rothman, 1990; Molz et al., 2008). "Theory of excitatory toxicity" believes that an important cause of neuron damage is due to a massive Glu release, impaired Glu reuptake and activates EAA receptors in the postsynaptic membrane Hagberg and Lehmann, 1985; Rossi et al., 2000). In addition a severe toxic effect of Ca^{2+} accumulation in the cerebral ischemia causes a series of pathological reactions, and promotes secondary cerebral ischemic injury as the final common pathway of necrotic death. The reduction in the release of Glu and Ca^{2+} can inhibit the excitatory toxicity of Glu, protect neurons from ischemic injury, and then reduce

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brain damages (Randall and Thayer, 1992; Stout et al., 1998).

Recent studies show that the activation of astrocytes and the release of excess glutamate with cerebral ischemia participates in the abnormal microenvironment and damage to neurons (Rossi et al., 2000; Trendelenburg and Dirnagl, 2005). Glutamate clearance mainly depends on reversed uptake of glutamate transporter (Chao et al., 2010). Five kinds of excitatory amino acid transporter (EAATs) include EAAT1 (called GLAST), EAAT2 (also known as GLT-1), EAAT3, EAAT4 and EAAT5. GLT-1 is mainly distributed in astrocytes as the major transporter of excitatory amino acid (Rao et al., 2001a). Normally GLT-1 can mediate the Glu uptake by glial cells to maintain the extracellular concentration of Glu. During cerebral ischemia, the function of GLT-1 is impaired, which causes a lot of accumulation of Glu in synaptic space (Rao et al., 2001b). The expression level of GLT-1 is associated with Glu increase in abnormal microenvironment. The abnormal increase of Glu leads to excessive activation of NMDA-receptor (NMDAR), and causes the influx of Ca^{2+} through Ca^{2+} channels and NMDAR channels, resulting in intracellular Ca^{2+} overload and a series of enzymatic activities which are related to cell toxicity (Bano and Nicotera, 2007; Yao and Haddad, 2004). The expression of NMDAR can reflect the abnormal condition of the microenvironment (Khaksari et al.,2012; Zhang et al., 2012). Cytochrome-C (Cyt-C) is an electron carrier of the mitochondrial respiratory chain, which is difficult to enter the cytoplasm through mitochondrial outer membrane. The accumulation of Ca^{2+} and Glu with abnormal microenvironment can promote release of mitochondrial Cyt-C, and activate caspase cascade which causes mitochondrial stress and regulates apoptosis directly (Zhao et al., 2010), and which is the key factor of cerebral ischemia injury (Dave et al., 2011; Zhou et al., 2011).

Therefore, in the abnormal microenvironment caused by the release of excitatory amino acids and overload of Ca^{2+} after cerebral ischemia, the expression level GLT-1, NMDAR and the release of mitochondrial Cyt-C provide the key link to promote neuronal damage.

Panax notoginseng saponin (PNS) is a Chinese herb that is widely used in traditional Chinese medicine to promote fibrinolytic function, decrease fibrinogen and antiplatelet aggregation so that it has a positive impact to prevent occurrence of cerebral vascular disease (Ng, 2006). Ginsenoside Rb1 and ginsenoside Rg1 are major active components of PNS which decrease the blood viscosity (Zheng et al.,2008). Ginsenoside Rb_1 has a significant effect on improving stress with a longer half-life and higher bioavailability, while ginsenoside Rg_1 can improve learning and memory with a shorter half-life (Wang et al., 2011; Xu et al., 2014). Ginsenoside Rb1 blocks Ca^{2+} channel and has effect of anti oxygen free radicals (Zhong et al., 1995). However, although there are a lot of researches on PNS, the molecular mechanism of ginsenoside Rb1 in the improvement of abnormal microenvironment after brain ischemia is poorly studied.

As the hippocampal neurons are most sensitive to ischemia and abnormal microenvironment, the CA1 region of hippocampus was selected for the region of interest (ROI) in this study. Therefore, we used rat hippocampal abnormal microenvironment model caused by microperfusion of glutamate and calcium, and ischemic microenvironment model caused by photothrombosis. The changes of rCBF in the hippocampal CA1 region, the expression of Cyt-C, NMDAR and the release of mitochondrial GLT-1 and the changes of cell ultrastructure were monitored. The efficacy of Ginsenoside Rb1 and the mechanism were investigated using a clinical drug nimodipine as a positive control.

2. Materials and methods

2.1. Animal methods

Adult Sprague-Dawley (SD) male rats (270–330 g, n=96) were used to implement photothrombosis for stroke or microperfusion. They were ordered or bred and acclimatized in Laboratory Animal Care qualified facility for up to 1 week before surgery. All animal handling and surgical procedures were approved by the Animal Research Ethics Committee of Kunming Medical University.

The rats after modelling were randomly assigned to following groups: A: sham group, B: model control, C, D, E: ginsenoside Rb1 groups with high, medium and low dose respectively, F, G, H: nimodipine at high, medium and low dose respectively.

2.2. Model preparation and drug delivery

Stroke model in rats by photothrombosis (Cerebral ischemia model): Rats were under fasting for 12hs before surgery. Cortical brain lesions were induced by photothrombosis according to Watson and Prado (2009) with slight modifications. They were intraperitoneally (i.p.) injected with 3% pentobarbital sodium. A midline scalp incision was made and the scalp was retracted laterally to expose the skull. Freshly prepared Bengal Rose solution (Sigma®, 0.2 mg/100 g per animal) was injected into the tail vein. Rats were then transferred within 1 min to a stereotaxic frame and their brains were exposed (through an intact skull) to Red laser light (Roithner Laser, 50 mW output) for 20 min. The center of the light beam was focused on the right cortex (lamda suture anterior 4 mm, 3 mm laterally (R) (Paxinos and Watson, 1998). After 4 h, the ginsenoside Rb1 at 1% was intraperitoneally given (high dose group 100 mg/kg, medium dose group 50 mg/kg, low dose group 25 mg/kg). After 4 h, the nimodipine was intraperitoneally given (high dose group 1 mg/kg, medium dose group 0.5 mg/kg, low dose group 0.25 mg/kg). Cerebral ischemia sham group rats were injected with the equal volume of normal saline without iv rose bengle.

Microperfusion model: Rats were under fasting for 12 h before surgery. Brain stereotactic positioning system was used to position the mouse in anesthesia, then microperfusion probe was inserting in rat brain at position (lamda point anterior 4 mm, 3 mm laterally (R),H=4 mm) . Pump constant microperfusion system was used to perform microperfusion of 0.1 mol/LGlu and 0.0002 mol/L$CaCl_2$ in the right hippocampus at perfusion velocity of 10 μl/min for 30 min, to anthropogenic change microenvironment of hippocampus and prepare microperfusion model. After 4 h, the ginsenoside Rb1 at 1% was intraperitoneally given (high dose group 100 mg/kg, medium dose group 50 mg/kg, low dose group 25 mg/kg). After 4 h, the nimodipine was intraperitoneally given (high dose group 1 mg/kg, medium dose group 0.5 mg/kg, low dose group 0.25 mg/kg). In the microperfusion sham group, just operation was performed without microperfusion. The model group and the sham group were injected with an equal volume of normal saline solution after 4 h of operation.

2.3. Determination of rCBF in hippocampus

After 24 h of surgery, the animals were i.p. injected with 3% pentobarbital sodium. Stereotactic apparatus (TSE Systems, Bad Homburg, D) was used to fix the skull, and laser Doppler flowmetry (PeriFlux System 5000) contact probe was set to hippocampus (lamda point anterior 4 mm, 3 mm laterally (R),H=4 mm). After the cerebral blood flow is stable, Perisoft which was supported by Perimed was used to record the blood flow curve. The rCBF of hippocampus CA1 region was monitored for 24 h, with equilibrium determination for 5 min as the average, to observe the changes of rCBF in hippocampal CA1 region.

2.4. Electron microscopic examination

After rCBF measurement, the hippocampus CA1 region was rapidly separated. Then separated hippocampus CA1 region was fixed in 1% osmic acid for 24 h. Then they were observed in electron microscope of EJM-100CX.