Neuroprotective effect of ginsenoside Rb1 on glutamate-induced neurotoxicity: With emphasis on autophagy

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ABSTRACT

Ginsenoside Rb1 has been demonstrated with neuroprotective effects, but the mechanisms remain unclear. This study aimed to probe the effects and mechanisms of ginsenoside Rb1 on activation of autophagy in glutamate-injured neurons. Ginsenoside Rb1 of exponential concentrations (1.2, 12, 120 μM) or autophagy inhibitor 3-methyladenine (5 mM) was added to culture medium for cortical neurons after being treated with glutamate. Cell viability was measured by MTT assay. Autophagosomes formation was observed with transmission electron microscope. Autophagy marked protein LC3 was detected with immunofluorescence and visualized under laser confocal microscopy. Changes of autophagy related protein Beclin-1 were measured with Western blot. We found that ginsenoside Rb1 protected cortical neurons from glutamate-induced cell injury. Autophagy was activated after glutamate treatment, with both autophagosomes and punctate LC3 increased significantly compared with control. Beclin-1 was elevated in glutamate-treated cells. Formation of autophagosome and punctate LC3 was attenuated by ginsenoside Rb1. The level of Beclin-1 in ginsenoside Rb1 treated cells was simultaneously decreased compared with glutamate-treated cells. These results suggested that inhibition of autophagy could be responsible for neuroprotective effects of ginsenoside Rb1 in glutamate-induced injury. Down-regulation of Beclin-1 may play an important role in this process.

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Ginseng has been utilized as an invigorator for thousands of years in Far East. Pharmacological studies indicated that ginsenosides are the main active components of ginseng. More than 40 kinds of ginsenosides have been identified to date. Ginsenoside Rb1 (GRb1) has garnered much research interest, and has been proved with effects in protecting neurons from glutamate-induced injury in vitro [16,28]. As a result, the possibility of treating some central nervous system (CNS) disorders with GRb1 has been proposed [11,28].

Glutamate-induced neuron toxicity has been regarded as an ideal model for studying neurological diseases [4,7,29], because it may mimic the pathogenic process of Alzheimer and other neurodegenerative diseases [13]. The mechanisms are related to increased calcium inflow, accumulative reactive oxygen species (ROS), impaired mitochondrial function and the initiation of apoptotic and autophagic process [6,13,24,26].

Autophagy is a physiological process for degrading abnormal cytosolic macromolecules and organelles. It contributes essentially in maintaining cell metabolism and homeostasis [19]. Macroautophagy (referred as autophagy in this paper) is the most common form of autophagy. It is characterized by formation of cup-shaped preautophagosomal double membrane structure which surrounds cytoplasmic ingredients and closes to form the autophagosome. However, autophagy has also been suggested to play a role in the development of neurodegenerative diseases. It can trigger a unique cell death distinct from apoptosis and necrosis [15,19]. Recent evidences suggested that autophagy plays an important role in glutamate-mediated neuron toxicity [2,15,21], but the mechanism remains unclear.

The neuroprotective mechanisms of GRb1 against glutamate-induced neurotoxicity were related with lipid peroxidation, reducing calcium influx and antagonizing apoptosis [5,20]. To our knowledge, the effects of GRb1 on autophagy or autophagic cell death in glutamate-injury have not been investigated so far. In this study, we assessed autophagy in glutamate-injured neurons as well as the effects of GRb1 on this injury.

Standard GRb1 was obtained from Zelang Biotechnology (Nanjing, China). Dulbecco's Modified Eagle's Medium (DMEM) was obtained from Invitrogen (Carlsbad, CA, USA). The monoclonal rabbit-anti-LC3 and TRITC-goat-anti-rabbit IgG came from Cell Signalling Test (CST), and monoclonal mouse-anti-β-actin IgG was purchased from Santa Cruse Biotechnology (Santa Cruz, CA, USA).
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