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Glycyrrhizae Radix and isoliquiritigenin, its active compound, inhibit Aβ (25-35)-induced Neuronal Toxicity in cultured cortical neurons

Hong Gyu Lee, Yeon Hee Seong*

College of Veterinary Medicine, Chungbuk National University

Objectives

Alzheimer's disease (AD), the most common form of the senile dementia, is a progressive neurodegenerative disorder that deprives the patient of memory and eventually leads to death. Although the pathological mechanism of AD is uncertain, it is characterized by its two histopathological hallmarks, senile plaque and neurofibrillary tangles. Amyloid β protein (Aβ), a major constituent of senile plaque, is a 39-43 amino acid fragment derived from amyloid precursor protein and is thought to be a significant factor in developing AD. Many in vitro studies indicated that Aβ mediates excessive increases in intracellular calcium level ($[Ca^{2+}]_i$) and the subsequent generation of reactive oxygen species (ROS). Excessive Ca^{2+} influx induced by Aβ disrupts intracellular Ca^{2+} homeostasis and is related to mitochondrial dysfunction and oxidative stress followed by apoptotic neuronal death. It, thus, is suggested the blockades of these pathways are main interest for prevention and treatment of AD. *Glycyrrhizae* Radix (GR), the root of *Glycyrrhiza uralensis* (Lycophodiaceae), one of the most famous medicinal plants in traditional Chinese medicine, has been widely used in oriental medicine due to its diverse pharmacological properties including antidepressant, anti-inflammatory and anti-oxidant effects. Isoliquiritigenin, a flavonoid constituent isolated from GR, has various pharmacological effects such as anti-stroke, antioxidant and anti-inflammatory effect. The aim of our study was to determine whether GR and isoliquiritigenin protect neuronal cells from Aβ (25-35)-induced damage using primarily cultured neurons.

Materials and Methods

Primary cortical neuron cultures were prepared using embryonic day 15 to 16 SD rat fetuses. Neurotoxicity experiments were performed on neurons after 3-4 days in culture. Cultured neurons were treated with 10 μM Aβ (25-35) in serum-free DMEM at 37℃ for 36 h to produce neurotoxicity. MTT assay and Hoechst 33342 staining were performed to measure neuronal death induced by 10 μM Aβ (25-35). Changes in $[Ca^{2+}]_i$ and ROS were measured with fluorescent dyes, Fluo-4 AM and H_2DCF-DA, respectively, using a laser scanning confocal microscope. Western blotting was performed to examine apoptotic protein expression.

주저자 연락처 (Corresponding author) : 성연희 E-mail : vepharm@cbnu.ac.kr Tel : 043-261-2968

Results

GR over the concentration of 10 to 50 μg/ml prevented the Aβ (25-35) (10 μM)-induced neuronal cell death, as assessed by a MTT assay and Hoechst 33342 staining. GR also significantly inhibited Aβ (25-35)-induced elevation of $[Ca^{2+}]_i$, generation of ROS and apoptotic protein changes. Isoliquiritigenin (1-20μM), isolated from GR as an active component, also inhibited Aβ (25-35)-induced neuronal death, elevation of $[Ca^{2+}]_i$ and ROS generation in cultured cortical neurons, suggesting that neuroprotective effect of GR may be, at least partly, attributable to this component. In conclusion, it is suggested that GR has a possible therapeutic role for preventing the progression of neurodegenerative disease such as AD.

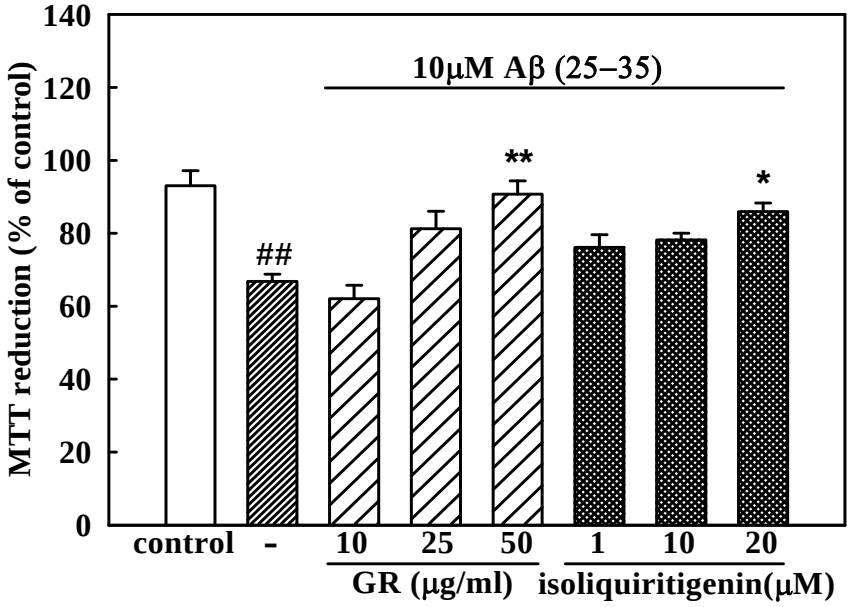

Fig. 1 Inhibitory effects of GR and isoliquiritigenin on Aβ (25-35)-induced neurotoxicity in cultured cortical neurons.