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과산화수소로 유도된 배양된 흰쥐 대뇌신경세포의 손상에 대한 감초 (Glycyrrhizae Radix)의 억제 효과

충북대학교 : 경장빈, 이홍규, 성연희*

Neuroprotective effect of Glycyrrhizae Radix on neuronal injury induced by hydrogen peroxide in cultured rat cortical neurons

College of veterinary medicine, Chungbuk National University
Jang been Kyung , Hong kyu Lee and Yeon hee Seong*

Objectives

Glycyrrhizae Radix (GR), the root of *Glycyrrhiza uralensis* (Lycophodiaceae), has been widely used in oriental medicine for having various pharmacological activities including anti-depressant and anti-inflammatory effects. Many compounds showing various bioactivities have been isolated from GR, which were reported to have anti-dementia and anti-ischemic effects in animal models. However, the neuroprotective effects of GR have not been studied in *in vitro*. Hydrogen peroxide (H_2O_2), which is involved in brain ischemic injury and neurodegenerative diseses, induces sustained elevation of intracellular calcium concentration ($[Ca^{2+}]_i$), stimulation of NMDA receptor triggered by glutamate release, and subsequent generation of reactive oxygen species (ROS). In the present study, the neuroprotective effect of GR against H_2O_2-induced oxidative neuronal cell damage was examined in primarily cultured rat cortical neurons.

Materials and Methods

Primary cortical neuronal culture were prepared from the forebrains of 16-day-old fetuses. The measurement of neuronal cell death induced by 100 μM H_2O_2 was performed by the 3-[4,5-dimethlthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay and scoring the number of apoptotic nuclei by Hoechst 33342 staining. $[Ca^{2+}]_i$ increase and ROS generation induced by 100 μM H_2O_2 were detected with fluorescent dye, Fluo-4 AM and H_2DCF-DA, respectively, using laser scanning confocal microscope.

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

Results

In cultured rat cortical neurons, 100 μM H_2O_2 reduced MTT reduction rate, indicating neuronal cell death. GR (25, 50 and 100 μg/ml) significantly inhibited the H_2O_2-induced decrease of MTT reduction rate. H_2O_2-induced apoptosis was also blocked by the treatment with GR. H_2O_2 induces elevation of $[Ca^{2+}]_i$ and generation of ROS in cultured rat cortical neurons, which were bloked by pretreatment of GR. These results suggest that GR has protective effect on H_2O_2-induced neurotoxicity. In conclusion, neuroprotective effect of GR against H_2O_2-induced oxidative damage in cultured rat neurons might be attributable to antioxidant activity or inhibition of Ca^{2+}influx.

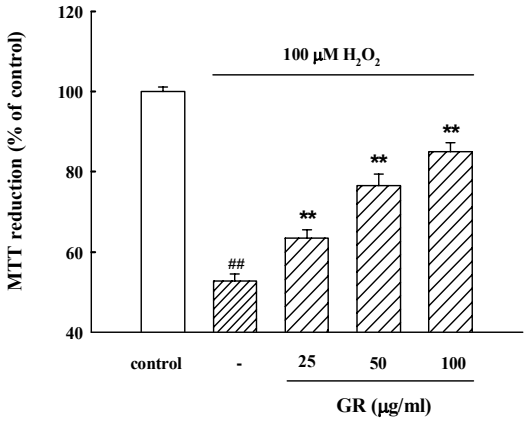

Fig. 1. Inhibitory effect of GR on H_2O_2-induced neuronal death in cultured cerebral cortical neurons. Results are expressed as mean ± S.E.M. of the data obtained from three independent experiments. $^{\#\#}$ $p<0.01$ vs control. * $p<0.05$, ** $p<0.01$ vs H_2O_2 (100 μM) (Tukey's test).

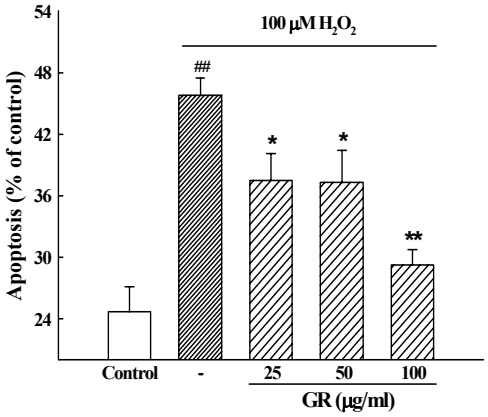

Fig. 2. Inhibitory effect of GR on apoptosis of cultured cerebral cortical neurons as measured by Hoechst 33342 staining. Results are shown as apoptotic cells as a percentage of total number of cells and expressed as mean ± S.E.M. of the data obtained from three independent experiments. $^{\#\#}$ $p<0.01$ vs control. * $p<0.05$, ** $p<0.01$ vs H_2O_2 (100 μM) (Tukey's test).