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Methanol extract of Smilacis chinae rhizome inhibits NMDA-induced neurotoxicity in cultured cortical neurons and cerebral ischemia in rats

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Objectives

Smilax has various pharmacological effects including antiiflammatory, anticancer and antioxidant activity. We previously reported that methanol extract of *Smilacis chinae* rhizome (SCR) from Smilax china L. (Liliaceae) inhibits Aβ (25-35)-induced neurotoxicity in cultured rat cortical neurons. NMDA receptor activation with the excessively released glutamate in many neurodegenerative disease such as Alzheimer disease and stroke has been considered as critical factor of neuronal cell death. The present study aims to investigate the effect of SCR on N-methyl-D-aspartate (NMDA)-induced neurotoxicity in cultured rat cortical neurons. Furthermore, we examined the inhibitory effect of SCR on brain infarct generation related to cerebral ischemia using middle cerebral artery occlusion model, which is very useful for the drug screening for neurodegenerative disease like stroke in SD rats.

Materials and Methods

Primary cortical neuronal cultures were prepared from the forebrains of 15-day-old fetuses from pregnant SD rats. The 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay and the number of apoptotic nuclei, evidenced by Hoechst 33342 staining, were tested for the measurement of neuronal cell death 24 h after the treatment with 1 mM NMDA for 30 min. $[Ca^{2+}]_c$ and ROS generation induced by 10 μM Aβ (25-35) were detected with fluorescent dye, Fluo-4 AM and H_2DCFDA, respectively, by laser scanning confocal microscopy. Cerebral ischemia was produced by insertion of silicone coated nylon suture through external carotid artery to internal carotid artery about 20 mm in isoflurane anaesthetized SD male rats (300g). Occlusion time was 3 h and reperfusion time was 24 h. SCR were orally administed at 0.5 h before and 1 h after occlusion and reperfusion. Brain was isolated and cut by 2 mm 24 h after reperfusion and the brain slices were stained by 2% triphenyltetrazolium chloride (TTC), and infarct volume was measured by graphic analyzer.

Results and discussion

CSR, over a concentration range of 5 to 50 μg/ml, inhibited NMDA (1 mM)-induced neuronal cell death (Fig. 1). Pretreatment of CSR (50 μg/ml) inhibited NMDA (1 mM)-induced elevation of cytosolic calcium concentration ($[Ca^{2+}]_c$), and generation of

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reactive oxygen species (ROS). Furthermore, in middle cerebral artery occlusion model, SCR (30 and 50 mg/kg) potentially reduced the transient ischemia-induced cerebral infarct volume (Fig. 2). Most of the previous hypotheses dealing with neurodegenerative diseases have invoked abnormal release and/or decreased uptake of the excitatory amino acid glutamate as playing a key role in the process of excitotoxicity. Therefore, these findings suggest that SCR has a possible therapeutic role in neurodegenerative diseases such as Alzheimer's disease and stroke.

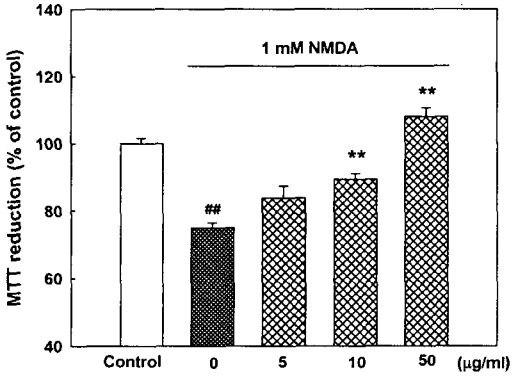

Fig. 1. Inhibitory effect of SCR on NMDA-induced neuronal cell death in cultured cortical neurons.

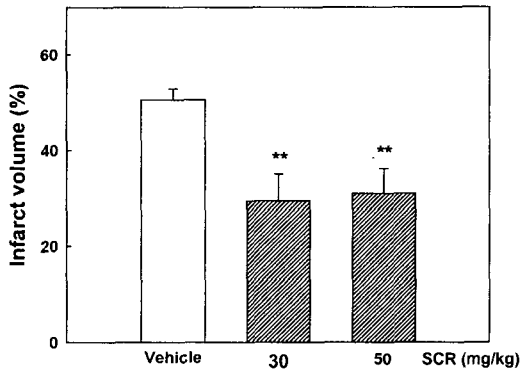

Fig. 2. Inhibitory effect of SCR on cerebral ischemia induced brain infarction.