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Anti-ischemic effect of *Ilex latifolia* : *in vitro* and *in vivo*

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*Ilex latifolia*의 항허혈 효과 : *in vitro* and *in vivo*

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Objectives

Ischemic stroke, one of the leading causes of death and long-lasting disability, results from a transient or permanent reduction in cerebral blood flow in a major brain artery. Increased extracellular glutamate levels and subsequent excitotoxicity are thought to be one of the major pathological factors leading to neuronal death in stroke. The leaves of *Ilex latifolia* (IL), which is one original species of ku-ding-cha, is generally consumed in southern china as a tea-like beverage. It has been demonstrated to possess coronary vasodilative actions and are used in the treatment of coronary heart disease and myocardial infarction. In the present study, we investigated protective effect of IL on glutamate-induced neurotoxicity in cultured neurons and anti-ischemic effect of IL on ischemia induced by Middle Cerebral Artery occlusion (MCAo)/reperfusion.

Materials and Methods

Materials

Ilex latifolia (IL) : ethanol extract of *Ilex latifolia*, glutamate, SD rats

Methods

Neuronal cells, cultured from 16-day-old fetuses of SD rats, were treated by glutamate (8h). Viability of cultured cells was measured by 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay and Hoechst 33342 staining. Glutamate-induced elevation of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) and generation of reactive oxygen species (ROS) were measured by fluorescence dyes using laser scanning confocal microscopy. Microglial cells, cultured from one-day-old SD rat pups were treated by

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lipopolysaccharides(LPS). LPS-induced Nitric oxide (NO) production was determined by the Griess reaction. Cerebral ischemic injury was induced by 2-h MCAo and 24-h reperfusion in SD rats.

Results

IL (10-100 μg/ml) inhibited glutamate-induced neuronal cell death. Glutamate- induced elevation of $[Ca^{2+}]_c$ and generation of ROS were inhibited by IL. IL (50-100 μg/ml) reduced LPS-induced NO production. Reduced extracellular glutamate may reduce NO production. IL (200 mg/kg) prevented cerebral ischemic injury induced by 2-h MCAo and 24-h reperfusion. Ischemic rats showed neurological signs, such as circling movement and decreased grip of contralateral forelimb and IK (200 mg/kg) significantly prevented such behavioral deficits. IL (200 mg/kg) treatment significantly decreased the histological changes observed following ischemia. In conclusion, the present study provides the pharmacological basis of IL as a promising agent for the treatment of neurodegenerative disease including stroke.

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

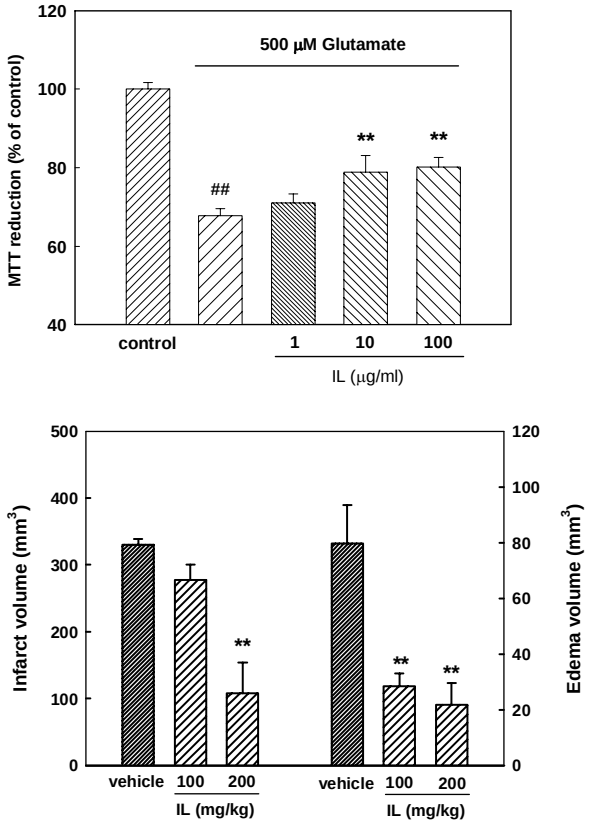

Fig 2. Inhibitory effect of IL on ischemia-induced infarct and edema formation in rats.