

Geniposide from *Gardenia jasminoides* Attenuates Neuronal Cell Death in Oxygen and Glucose Deprivation-Exposed Rat Hippocampal Slice Culture

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Geniposide from *Gardenia jasminoides* protected neuronal cells from damage in oxygen and glucose deprivation-exposed hippocampal slice culture. Geniposide showed a greater protective effect on the granule cell layer than on the pyramidal cell layer including CA 1 and CA 3. On the basis of the experimental results, geniposide may be a therapeutic agent for ischemia in patients.

Key words geniposide; *Gardenia jasminoides*; OGD; hippocampal slice culture

Since the brain needs approximately 20% of inhaled oxygen for physiological function, interruption of blood flow causes serious harm to the brain. In particular, damage of the CA 1 region in the hippocampus, the most vulnerable to oxygen deficiency, leads to fatal impairment of learning and memory.¹⁾ Experimental *in vitro* and *in vivo* models have been developed to demonstrate the toxicity mechanism mediated by ischemia, and to select neuroprotective agent candidates. Because of its relevance to animal models, easy handling and less expensive cost, slice cultures have been highlighted as an experimental model.^{2,3)}

The fruits of *Gardenia jasminoides* ELLIS (Rubiaceae) are an oriental medicine used in the treatment of inflammation, jaundice, headache, edema, fever, hepatic disorders and hypertension.⁴⁾ Regarding the pharmacological activities of *G. jasminoides* and its ingredients, it has been reported to have an anti-angiogenic effect and anti-thrombotic effect.^{5,6)}

In this study, we investigated the effect of geniposide isolated from *G. jasminoides* on oxygen and glucose deprivation (OGD)-induced neuronal cell death in a rat hippocampal slice culture system.

MATERIALS AND METHODS

Plant Material The fruits of *G. jasminoides* were purchased from a local market in Seoul, Korea in 2004. The fruits were authenticated by Prof. H. J. Chi, Seoul National University, Seoul, Korea. The voucher specimen (No. KTNG-5213) of the plant material was deposited at the Herbarium of KT&G Central Research Institute, Korea.

Isolation of Geniposide The air-dried powdered fruits (2.0 kg) of *G. jasminoides* were extracted three times with MeOH. The resultant extracts were combined and concentrated under reduced pressure to afford a residue (751 g). The MeOH extract was suspended in water, and then fractionated successively with equal volumes of *n*-hexane, EtOAc and *n*-BuOH, leaving a residual aqueous fraction. Each fraction was evaporated *in vacuo* to yield the residues of *n*-hexane (201 g), EtOAc (38 g), *n*-BuOH (210 g) and water-soluble

(308 g) fractions, respectively. A portion of the *n*-BuOH fraction was chromatographed on a silica gel column (7×60 cm), and eluted with a gradient of CHCl₃–MeOH to afford geniposide.

Geniposide: FAB-MS *m/z*: 389 [M+H]⁺; IR ν_{\max} (KBr) cm⁻¹: 3428 (–OH), 1712 (ester), 1639 (conjugated C=C), 1074 (C–O); ¹H-NMR (400 MHz, DMSO): δ 7.47 (1H, s, H-3), 5.67 (1H, br s, H-7), 5.12 (1H, d, *J*=7.3 Hz, H-1), 4.52 (1H, d, *J*=7.8 Hz, H-1'), 4.13 (1H, br d, *J*=15.0 Hz, H-10a), 3.96 (1H, br d, *J*=15.0 Hz, H-10b), 3.63 (3H, s, OCH₃), 3.15 (1H, m, H-5), 2.69 (1H, br d, *J*=16.1 Hz, H-6a), 2.62 (1H, br t, *J*=7.7 Hz, H-9), 2.03 (1H, br d, *J*=16.1 Hz, H-6b); ¹³C-NMR (100 MHz, DMSO): δ 167.1 (C-11), 151.7 (C-3), 144.1 (C-8), 125.6 (C-7), 111.0 (C-4), 98.6 (C-1'), 95.8 (C-1), 77.4 (C-3'), 76.7 (C-5'), 73.4 (C-2'), 70.0 (C-4'), 61.0 (C-6'), 59.5 (C-10), 51.2 (OCH₃), 45.9 (C-9), 38.1 (C-6), 34.6 (C-5).

Rat Hippocampal Slice Culture Hippocampal slices were prepared and cultured according to the modified interface culture method. Sprague–Dawley rats (5–8 d old) were decapitated. The hippocampus was isolated and dorsal halves were cut in transverse sections at 400 μ m using a McIlwain tissue chopper (Mickle Laboratory Engineering Co., Surrey, U.K.). The six tissue slices were placed in random order on an insert membrane (0.4 μ m in pore size, 30 mm in diameter, Millipore Co., Bedford, MA, U.S.A.). The inserts were transferred to 6-well culture trays, where each well contained 1 ml culture medium composed of 50% α -MEM, 25% HS

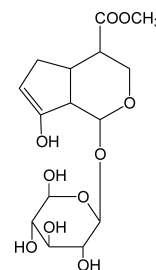


Fig. 1. Structure of Geniposide from *Gardenia jasminoides*

and 25% HBSS supplemented with 25 mM D-glucose. The medium was changed every 3 d, and experiments were carried out after 14 d.

Oxygen and Glucose Deprivation Subsequently, the culture medium of the hippocampal slices was replaced with Ischemic Balanced Salt Solution (IBSS: 143.4 mM NaCl, 5 mM HEPES, 5.4 mM KCl, 1.2 mM MgSO₄, 1.2 mM NaH₂PO₄, 2 mM CaCl₂) in the presence or absence of geniposide. OGD was conducted in a chamber containing an anaerobic gas mixture (95% N₂, and 5% CO₂) for 40 min. OGD was terminated by the removal of IBSS and the addition of serum-free medium containing 7.5 μM of PI with or without geniposide. The cultures were then incubated in a CO₂ incubator at 37 °C for 48 h.

Evaluation of Hippocampal Neuronal Cell Death Cell death was assessed using the fluorescent exclusion dye propidium iodide (PI), by which fluorescence was excited at 514 nm using a confocal laser scanning microscope (Carl Zeiss, LSM 510, Germany). The digital photos were analyzed directly in a public domain NIH image program (version 1.29). Cell death area of the 40 min OGD-exposed hippocampal slice was considered 100%. Compared with this 100% value, cell death in geniposide-treated hippocampal slices was calculated.

Statistical Analysis The data is presented as the mean ± S.E.M. from three independent experiments. Statistical comparison between the different treatments was done by one-way ANOVA followed by Turkey's test. Differences with a *p* value of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Treatment of OGD-exposed hippocampal slices with geniposide ameliorated the neuronal cell death of both the pyramidal (CA 1 plus 3 region) and granular cell layer (dentate gyrus region). When neuronal cell death in 40 min OGD-induced slices was set to 100%, cell death area in the pyramidal area was reduced to 90.6 ± 18%, 79.6 ± 20.4%, 26.5 ± 1.8% and 43.3 ± 9.9% upon treatment with 1, 10, 50, and 100 μM of geniposide, respectively. In the case of the granular cell layer, the cell death area was 81.2 ± 1.4% and 59.7 ± 1.7% upon treatment with 1 and 10 μM of geniposide, respectively. Fifty and 100 μM of geniposide completely blocked neuronal cell death in granular cell layer. Although Yamazaki *et al.* reported that geniposide could not protect hippocampal cells against β-amyloid-induced toxicity,⁷⁾ we found that geniposide attenuated cell death in OGD-exposed rat hippocampal slice culture. This discrepancy suggests the mechanism by which OGD induces cell death is different from cell death by β-amyloid. In this study, we found that geniposide protected neurons from OGD damage. However, there are two reasons the neuroprotective effect of genipin in our experimental model needs to be further investigated: 1) generally, herbal medicines are taken orally. Ingredients in herbal medicines are metabolized by various enzymes of, especially, the liver and intestine before distribution to each organ. In the case of geniposide, it has been reported that β-glucosidase of intestinal bacteria transforms geniposide into genipin, aglycone of geniposide.^{8,9)} Therefore, genipin, rather than geniposide, is believed to be absorbed in the body when

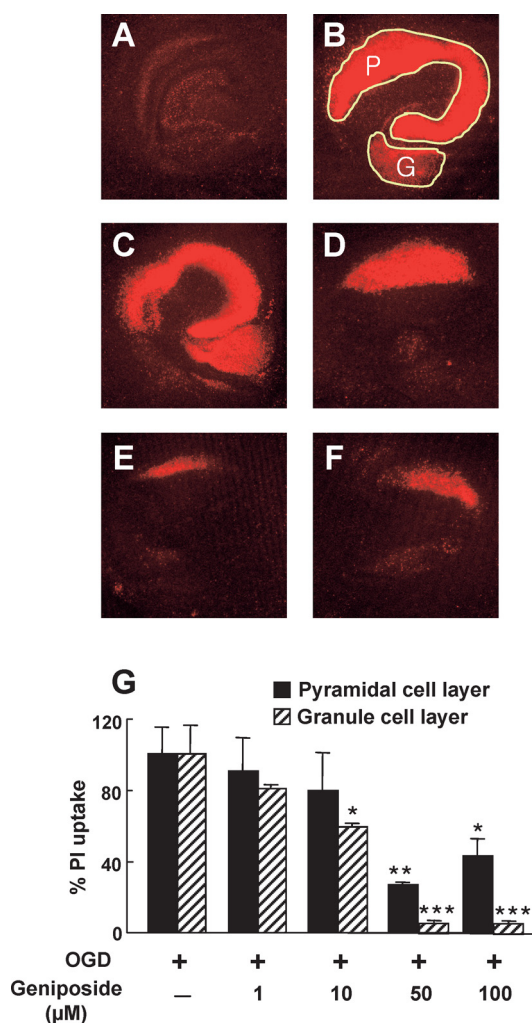


Fig. 2. Neuroprotective Effect of Geniposide in OGD-Exposed Rat Hippocampal Slice Culture for 40 min

Geniposide was used as a treatment both before and after OGD exposure to hippocampal slice culture for 40 min. Two days after wards, cell death was measured using PI uptake. The cell death area was analyzed using a public domain NIH image program. A: Normoxia, B: 40 min OGD and vehicle treatment, C: 40 min OGD and geniposide (1 μM), D: 40 min OGD and geniposide (10 μM), E: 40 min OGD and geniposide (50 μM), F: 40 min OGD and geniposide (100 μM), G: Cell death area of OGD and geniposide-exposed slice relative to that of only OGD-exposed slice. Three independent experiments were carried out. The data was expressed as a mean ± S.E.M. The asterisk indicates a significant difference from exposure to 40 min oxygen and glucose deprivation alone (**p* < 0.05, ***p* < 0.01, ****p* < 0.001, one-way ANOVA followed by Turkey's test).

G. jasminoides is administrated orally. In other words, genipin, not geniposide, is a major active compound of *G. jasminoides*. 2) Geniposide may be hydrolyzed into genipin and glucose when geniposide is used to treat the hippocampal slice tissue, and there is a possibility that genipin and (or) glucose prevent neuronal cell death. However, based on the previous result that a high concentration of geniposide (over 5 mM) exhibited a protective effect in hypoxia-exposed hippocampal slices,¹⁰⁾ glucose derived from geniposide might not be responsible for the inhibition of cell death. At present, we are carrying out experiments to demonstrate how well genipin protects neuronal cells and whether geniposide is hydrolyzed in OGD-exposed hippocampal slices. Overall, we found geniposide to be neuroprotective using OGD-exposed hippocampal slice culture. Previous experimental evidence has demonstrated that genipin showed an anti-inflammatory

effect, and it is assumed that neuroprotection by geniposide may be due to its anti-inflammatory effect. More investigation should be focused on the potency of geniposide and genipin as leading neuroprotective compounds.

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