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Short communication

Neuroprotective effect of wogonin in hippocampal slice culture exposed to oxygen and glucose deprivation

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Abstract

A poor supply of oxygen and glucose to the brain can cause severe brain damage. Therefore, neuroprotective drugs against ischemia need to be developed. In this study, wogonin, a flavone found in *Scutellaria baicalensis*, had a protective effect on neuronal cells damaged by oxygen and glucose deprivation in rat hippocampal slices in culture. In particular, the protective effect on the pyramidal cell layer was significant. On the basis of these experimental results, wogonin may be a therapeutic agent for treating ischemia in patients.

Keywords: Wogonin; Oxygen and glucose deprivation; Hippocampal slice culture; Neuroprotection

1. Introduction

The brain is an organ that requires a large amount of energy for its physiological functions. For this reason, the brain consumes 20% of inhaled oxygen. A poor supply of oxygen and glucose causes serious injury to neuronal cells. In particular, the hippocampus is the brain region the most vulnerable to hypoxia, and the loss of hippocampal neurons results in learning and memory impairments. Therefore, the development of neuroprotective drugs against ischemia is urgently required.

Scutellaria baicalensis is used in Korea to treat bacterial infection of respiratory and gastrointestinal tract, and diverse inflammatory diseases in Korea. Interestingly, recent experimental results have suggested that *S. baicalensis* has neuroprotective effects. *S. baicalensis* prevented neuronal cell death in the hippocampal CA 1 region induced by a four-vessel occlusion (Kim et al., 2001b) and attenuated apoptosis by inhibiting protein oxidation in a H_2O_2-treated neuronal cell line (Choi et al., 2002). These reports suggest that some compound(s) contained in *S. baicalensis* have neuroprotective effects. Flavones are believed to be the major biologically active agents. Among the flavones of *S. baicalensis*, wogonin, 5,7-dihydroxy-8-methoxyflavone, has been reported to show anti-oxidant, anti-inflammatory and anxiolytic effects (Gao et al., 1999; Wakabayashi and Yasui, 2000; Shen et al., 2002; Hui et al., 2002). However, the neuroprotective effect of wogonin remains unknown.

This study investigated whether wogonin protects neuronal cells from injury caused by oxygen and glucose deprivation in a hippocampal slice culture.

2. Materials and methods

2.1. Materials

α-Modified Eagle Medium (α-MEM), a penicillin/streptomycin solution, Hank's balance salt solution (HBSS) and horse serum were purchased from Gibco BRL (Grand Island, NY, USA). Wogonin was obtained from Waco Pure

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Chemicals (Osaka, Japan). Propidium iodide (PI), D-glucose, and all the other chemicals were purchased from the Sigma (St. Louis, MO, USA).

2.2. Organotypic hippocampal slice culture

Hippocampal slices were prepared and cultured according to a modified interface culture method (Noraberg et al., 1999). Sprague–Dawley rats (7 days old) were decapitated. The hippocampus was isolated and the dorsal halves were cut into 400-μm transverse sections using a Mcllwain tissue chopper (Mickle Laboratory Engineering, Surrey, UK). Six tissue slices were placed in random order on an insert membrane (0.4-μm pore size, 30 mm in diameter, Millipore, Bedford, MA, USA). The inserts were transferred to 6-well culture plates, each well of which contained 1 ml of culture medium composed of 50% α-MEM, 25% horse serum and 25% HBSS supplemented with 25 mM D-glucose. The medium was changed every 3 days and the experiments were carried out after 14 days.

2.3. Oxygen and glucose deprivation injury and wogonin treatment

After the culture medium of the hippocampal slices was replaced with an Ischemic Balanced Salt Solution (IBSS: 143.4 mM NaCl, 5 mM HEPES, 5.4 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 2 mM $CaCl_2$) in the presence or absence of wogonin, oxygen and glucose deprivation was achieved in a chamber containing an anaerobic gas mixture (95% N_2, and 5% CO_2) for 40 min and was terminated by removing the IBSS and adding serum-free medium containing 7.5 μM of PI with or without wogonin. The cultures were incubated in a CO_2 incubator at 37 °C for 48 h.

2.4. Cell death analysis

Cell death was assessed using a fluorescent exclusion dye, PI, in which fluorescence was excited at 514 nm using confocal laser scanning microscopy (Carl Zeiss, LSM 510, Germany). The digital photos were analyzed directly using a public domain NIH image program. Cell death in slices deprived of oxygen and glucose for 40 min was set to 100%. Cell death in the wogonin-treated hippocampal slices was calculated as a percentage of this value.

2.5. Statistical analysis

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

3. Results

3.1. Evaluation of neuronal cell death in oxygen and glucose deprivation injured hippocampal slice culture

In our experiment, cell death was observed after 35–40 min of deprivation of oxygen and glucose. Cell death was detected in the CA 1 region when the hippocampal slice was exposed for 35 min; however, cell death was observed in the whole hippocampal cell layer after exposure for 40 min (Fig. 1). In order to determine whether or not wogonin had a region-specific protective effect, the effect of wogonin on neuronal cell survival was observed in slices deprived of oxygen and glucose for 40 min.

3.2. Neuroprotective effect of wogonin in oxygen and glucose deprivation injured hippocampal slice culture

Cell death was detected 2 days after exposure of hippocampal slices for 40 min in the presence or absence of wogonin, using PI staining. As shown in Fig. 2, wogonin reduced the amount of neuronal cell death. In particular, wogonin had a significant protective effect on the pyramidal cell layer when compared to the granule cell layer (Fig. 2). In this study, 1 μM of wogonin could not protect against the cell death induced by oxygen and glucose deprivation. In addition, treat-

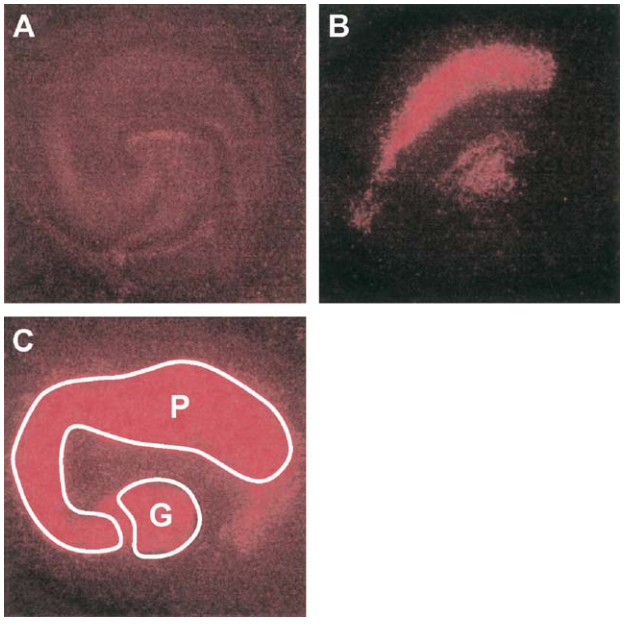

Fig. 1. Cell death in oxygen-and glucose deprived rat hippocampal slices. After hippocampal slices were exposed to oxygen and glucose deprivation for 35 or 40 min followed by culture for 48 h, cell death was detected using PI staining. (A) Normoxia, (B) 35 min oxygen and glucose deprivation, (C) 40 min oxygen and glucose deprivation. P and G in (C) indicates the pyramidal cell layer and granular cell layer, respectively.

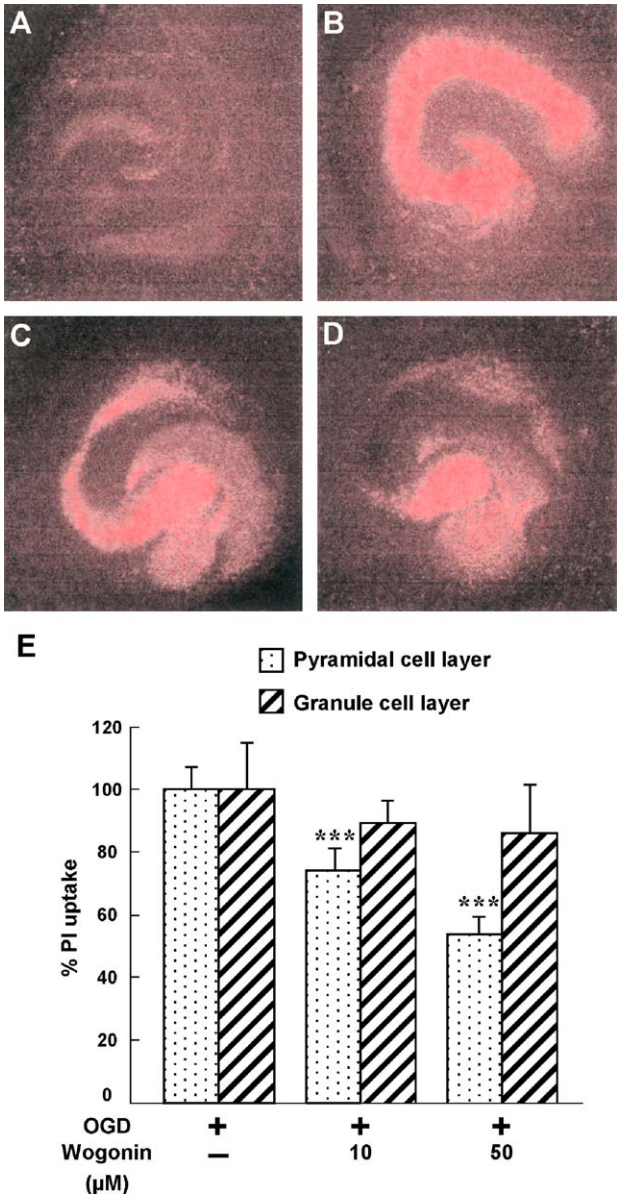

Fig. 2. Neuroprotective effect of wogonin on rat hippocampal slices in culture deprived of oxygen and glucose for 40 min. Wogonin was added before and after oxygen and glucose deprivation of hippocampal slices for 40 min. Two days later, cell death was assessed using PI uptake. The area of cell death was analyzed using a public domain NIH image program (version 1.29). (A) Normoxia, (B) 40 min oxygen and glucose deprivation and vehicle treatment, (C) 40 min oxygen and glucose deprivation and wogonin (10 μM), (D) 40 min oxygen and glucose deprivation and wogonin (50 μM). (E) Cell death of the oxygen- and glucose deprived hippocampal slices in the presence of wogonin ($n=6$). Three independent experiments were carried out. The data was expressed as means ± S.E.M. The asterisk indicates a significant difference from exposure to 40 min oxygen and glucose deprivation alone (***$p<0.001$, one-way ANOVA followed by Tukey's test).

ment with 100 μM of wogonin did not show a greater neuroprotective effect than that achieved with 50 μM of wogonin.

4. Discussion

Because of relevance to in vivo systems, much attention has been focused on the use of slices in culture for investigating pathophysiological events in the brain. (Frotscher et al., 1995; Gahwiler et al., 1997; Stoppini et al., 1991). Recently, a large number of agents have been examined for their neuroprotective effect against oxygen and glucose deprivation in hippocampal slices in culture. Lithium reduced the ratio of phosphorylated heat shock protein 27 (HSP 27) to total HSP 27 and afforded protection against neuronal cell death (Cimarosti et al., 2001). Treatment with lipoxygenase inhibitors reduced cell loss (Arai et al., 2001). In addition, 3-methyl-aminothiophene dicarboxylic acid (3-MATIDA), a metabotropic glutamate 1 (mGlu 1) receptor antagonist, reduced the oxygen and glucose deprivation-induced neuronal cell death (Moroni et al., 2002).

In this study, we found that wogonin counteracted the toxicity induced by oxygen and glucose deprivation in rat hippocampal slices in culture. However, its neuroprotective mechanism was not determined. Nonetheless, based on previous pharmacological studies of wogonin, there are several possible neuroprotective pathways that can explain the action of wogonin. The onset of hypoxia and hypoglycemia induces a pathological cascade of events, including ATP depletion, disruption of ion gradient homeostasis and release of glutamate, leading to neuronal cell death. Even if oxygen is re-supplied, generation of reactive oxygen species has a detrimental effect on neuron survival. Furthermore, recent experimental results demonstrated that post-ischemic inflammation is significantly associated with neuronal cell death. Overall, neuroprotective agents against ischemia are mainly thought to have three modes of action: an effect on glutamate metabolism and its receptor, inhibition of oxidative stress and reduction of inflammatory reaction. First, because we do not have enough information about wogonin on glutamate and its receptors to elucidate the neuroprotective effect of wogonin, we excluded this pathway. Second, in neurodegenerative diseases, including ischemia, reactive oxygen species have a significant deleterious effect on neuron survival. Therefore, antioxidants have been highlighted in neuroprotective drug development (Facchinetti et al., 1998; Gilgun-Sherki et al., 2001). Wogonin is reported to scavenge radicals and to inhibit lipid peroxidation (Gao et al., 1999; Shieh et al., 2000). It is assumed that the neuroprotection provided by wogonin can be partly attributed to its antioxidant effect. Third, it has been reported that *N*-(3-(aminomethyl)benzyl)acetamidine (1400W), an inducible nitric oxide synthase inhibitor, protects damaged cells in oxygen- and glucose deprived forebrain slices in culture, indicating that nitric oxide is harmful cells (Cardenas et al., 1998). In fact, it is generally accepted that nitric oxide and inflammatory cytokines, such as interleukin 1-β and tumor necrosis factor-α, are partly responsible for neurodegenerative disease, particularly, ischemia. Astrocytes and microglia

are a major source of these agents. Wogonin is reported to suppress the level of expression inducible nitric oxide synthase in activated C6 cells, in a rat astrocyte cell line and BV-2 cells, and in a murine microglia cell line (Kim et al., 2001a; Lee et al., 2003). Taken together, in this study, wogonin may have protected neuronal cells from the toxicity induced by oxygen and glucose deprivation by inhibiting the inflammatory reaction.

Interestingly, wogonin exhibited a region-specific neuroprotective effect in the pyramidal cell layer containing CA1 and 3 regions, indicating that the event leading to granular cell death in dendate gyrus is different from that in CA 1 and 3 regions. The exact protective pathways of wogonin in the CA 1 and 3 regions remain to be elucidated. In conclusion, based on the experimental results, wogonin appears to be a potential therapeutic agent against ischemia.

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