Wogonin Inhibits Ischemic Brain Injury in a Rat Model of Permanent Middle Cerebral Artery Occlusion

Jungsook Cho*,a and Hyeong-Kyu Leeb

aDepartment of Pharmacology, College of Medicine, Dongguk University; Gyeongju, Gyeongbuk 780–714, Korea; and  
bImmunomodulator Research Laboratory, Korea Research Institute of Bioscience and Biotechnology; Yuseong, Daejeon 305–600, Korea. Received May 19, 2004; accepted July 5, 2004

The present study evaluated the effect of wogonin, a flavonoid originated from the root of Scutellaria baicalensis GEORG, on focal ischemic brain injury in rats. Focal brain ischemia was induced by the permanent occlusion of middle cerebral artery (pMCAO) for 24 h with a silicone rubber cylinder inserted through the right internal carotid artery. We found that wogonin, intraperitoneally administered at a dosage of 20 mg/kg at 30 min before and 4 h after the surgery, reduced the pMCAO-induced infarct areas in the cerebral cortex as well as in the striatum. The total volume of infarction was significantly reduced by the treatment with wogonin. In addition, wogonin was found to significantly improve the pMCAO-induced behavioral deficits at 24 h after the surgery. Taken together, these results demonstrate that wogonin inhibits ischemic brain injury and improves behavioral dysfunction caused by pMCAO. These findings, along with previous reports demonstrating the neuroprotective effects of wogonin, provide strong pharmacological basis for the use of wogonin or Scutellaria baicalensis in the treatment of stroke.

Key words wogonin; neuroprotection; permanent middle cerebral artery occlusion; focal brain ischemia; neurological deficits

Flavonoids are a group of polyphenolic compounds found ubiquitously in plants. They have been shown to exhibit a variety of biological activities including anti-viral, anti-tumor, anti-inflammatory and vasodilatory actions.2,3) Wogonin (5,7-dihydroxy-8-methoxyflavone), a flavonoid originally derived from the root of Scutellaria baicalensis LABIATAE, is known to exert potent anti-inflammatory effects. It was shown to inhibit lipopolysaccharide-induced production of nitric oxide (NO) and prostaglandin E2 in macrophages.3,4) It was also reported to inhibit cyclooxygenase-2 expression and alleviate skin inflammation in mice.5) In addition to the anti-inflammatory action of wogonin, variable degrees of radical scavenging and antioxidant activities have been reported in many experimental systems.6–8) Wogonin was shown to scavenge hydroxyl radicals generated by Fenton reaction,6) inhibit xanthine oxidase activity and reduce cytochrome c.7) It also exhibited significant inhibition of NADPH-induced lipid peroxidation in rat brain cortex mitochondria.8)

Recently, additional pharmacological actions of wogonin have been reported in the central nervous system. It was found to attenuate the H2O2-induced oxidative stress in human neuroblastoma SH-SY5Y cells,9) and inhibit the neuronal damage induced in primary cultured rat cortical cells by various types of oxidative stress such as H2O2, xanthine/xanthine oxidase, and GSH depletion.9) It was also shown to inhibit the glutamate- or NMDA-induced excitotoxic damage in the cultured cortical cells.9) Moreover, wogonin was demonstrated to suppress the death of activated C6 rat glial cells by inhibiting NO production.10) The neuroprotective effects of wogonin have been further investigated in vivo using two experimental brain injury models.11) Lee et al.11) found that, in models of transient global ischemia induced in rats by 4-vessel occlusion and excitotoxic injury induced in mice by systemic kainate injection, wogonin attenuated the death of hippocampal neurons. They also found that the neuroprotective effect was associated with inhibition of the inflammatory activation of microglia.11)

To confirm and further characterize the neuroprotective effects of wogonin in vivo, this study evaluated its effects on ischemic brain injury using a permanent model of focal ischemia in rats. The animal model of focal brain ischemia is believed to be a useful experimental system that is closely related to stroke in humans.12) In this study, focal brain ischemia was induced in Sprague-Dawley (SD) rats by the permanent occlusion of middle cerebral artery (pMCAO) with a silicone rubber cylinder inserted through the right internal carotid artery. The effect of wogonin on behavioral dysfunction caused by the pMCAO was also assessed in this study.

MATERIALS AND METHODS

Animals Male SD rats weighing 260–270 g were purchased from Daehan Biolink (Chungbuk, Korea). Animals were maintained with Purina laboratory chow and water ad libitum in our animal facility with a 12 h light cycle at a controlled temperature (22 ± 2 °C) until used. All animal experiments were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Pharmacological Treatment Wogonin, obtained from Wako Pure Chemicals Ind. (Osaka, Japan), was dispersed in an aqueous solution of Tween 80 (5% w/v) and administered intraperitoneally at a dosage of 20 mg/kg at 30 min before and 4 h after the surgery. Rats in the control group received vehicle in the same volume and with the same time schedule as wogonin-treated animals.

Permanent Model of Focal Brain Ischemia Permanent focal brain ischemia model was induced by the occlusion of right MCA according to the method of Nagasawa and Kogure,13) with minor modifications. Briefly, rats were anesthetized and maintained with 1.5–3% isoflurane (Choongwae Pharmaceutical Co., Korea) in a mixture of N2O and O2 (7 : 3). Rectal temperature was maintained at 37.0 ± 0.5 °C with a heating pad throughout the surgical procedure. After a

* To whom correspondence should be addressed. e-mail: jscho@dongguk.ac.kr © 2004 Pharmaceutical Society of Japan
medial incision was made in the neck skin, the right common carotid artery was exposed, and a 17-mm-long silicone rubber cylinder, made with a 4-0 nylon surgical thread (Nitto Kogyo Co., Japan) by coating with silicone mixed with a hardener, was introduced from the bifurcation of the right internal carotid artery to the MCA. After the surgery, rats were allowed to recover from anesthesia. Rats that did not exhibit neurological deficits of left hemiparesis with upper extremity dormant and circling movement to the left were excluded from the study (<10%).

**Morphometric Measurement of Infarct Area in Rat Brain Slices** Rats were killed by decapitation after 24 h of pMCAO. The brains were carefully removed and placed in ice-cold saline, and then sliced into seven serial coronal sections of 2-mm thickness with a rat brain matrix (Harvard Apparatus, MA, U.S.A.) starting at 1 mm posterior to the anterior pole. Each slice was incubated for 60 min at 37 °C in saline containing 2% 2,3,5-triphenyl tetrazolium chloride (TTC, Sigma Chemical Co., St. Louis, U.S.A.), and immediately fixed by immersion in 10% phosphate-buffered formalin. The areas of infarct in the cortex and striatum were measured in each slice using a computerized image analysis system (Multiscan, CA, U.S.A.). The infarct volume in the cortex and striatum was respectively calculated by summation of the infarct area in each slice multiplied by the thickness of the slice.

**Assessment of Neurological Deficits** The degrees of neurological deficits were assessed by the method of Relton et al. At 4 h after surgery and immediately before the rats were killed. The assessment includes the following tests, as described.

(1) The degree of left forelimb flexion was scored between 0 and 3, while the rat was held in the air by the tail (0, no movement; 1, limited movement; 2, less extended or slower movement; 3, symmetrical movement). (2) While the rat was held in the air by the tail, the duration of left forelimb flexion was scored between 0 and 4 during a 10-s period (0, 8—10 s; 1, 6—8 s; 2, 4—6 s; 3, 2—4 s; 4, 0—2 s). (3) The symmetry of movement/forepaw outstretching was scored between 0 and 3, while the rat was made to walk along the bench on its forelimbs (0, left forelimb does not move; 1, left forelimb moves minimally and rat circles; 2, left forelimb outstretches less than right; 3, both forelimbs outstretch and rat walks normally). The total neurological score, ranging on a scale of 0—10, represents the cumulative score of the individual tests. The score of 10 reflects normal behavior.

**Statistical Analysis** Results are presented as the mean±S.E.M. obtained from nine rats in each group. Data were analyzed by either Student’s t-test or one-way analysis of variance (ANOVA) followed by Duncan’s test for multiple comparisons. p values less than 0.05 were considered to be statistically significant.

**RESULTS**

After 24 h of pMCAO, rat brains were removed, sliced into seven coronal sections, and immediately stained with TTC. The brain slices of the vehicle- or wogonin-treated rat are shown in Fig. 1. The normal area of the brain was stained deep red, whereas the infarct tissue was not stained by TTC, delineating the area of the ischemic damage (Fig. 1). The slices of the vehicle-treated rat brain exhibited hemispheric swelling and extensive brain infarcts involving both cortex and striatum (Figs. 1, the upper panel). In the rat treated with wogonin, the infarct area was markedly reduced in several slices (Fig. 1, the lower panel).

The areas of infarct in the cortex and striatum in each slice were measured using a computerized image analysis system. As illustrated in Fig. 2, wogonin reduced the infarct areas in the cortex as well as in the striatum. In comparison to the infarct areas in the control group, statistically significant reductions were observed in the slices located at 7—15 mm from the frontal pole of the wogonin-treated rats (Fig. 2). We then calculated the mean volumes of brain infarction in the cortex and striatum by summation of the infarct area in each slice multiplied by the thickness of the slice. The infarct volume was markedly and significantly reduced by wogonin treatment in both cortex and striatum (Fig. 3). The total infarct volume was reduced by 36.3% in the wogonin-treated rats, as compared to that in the vehicle-treated group.

Finally, behavioral deficits were assessed, before and 4 and 24 h after the pMCAO in both vehicle- and wogonin-treated rats, by neurological scoring of the degree and duration of forelimb flexion and symmetry of movement and forepaw outstretching, as described in the Materials and Methods. Before the pMCAO, the rats in both groups exhibited scores of 10, reflecting normal behavior (Fig. 4). At 4 h after the
Using the excitotoxic brain injury model induced by systemic injection of kainate in mice, the hippocampal neuronal cell death in both CA1 and CA3 areas was significantly attenuated by the pretreatment with wogonin. Based on these findings, it has been concluded that the neuroprotective effect of wogonin may be associated with inhibition of the inflammatory activation of microglia, which is believed to be a critical component of pathogenic inflammatory responses in neurodegenerative diseases.

In this study, we confirmed and further demonstrated the neuroprotective effect of wogonin using a permanent model of focal brain ischemia in rats. We found that wogonin reduced the pMCAO-induced infarct areas in the cortex as well as in the striatum. The total volume of infarction was reduced by 36.3%, as compared to that in the control group. Consistent with these observations, we found that behavioral deficits caused by pMCAO were significantly improved by wogonin treatment at 24 h after the surgery.

Lee et al. proposed that wogonin may protect neurons by inhibiting the inflammatory activation of microglia rather than by directly acting on neurons. Besides the anti-inflammatory neuroprotection by wogonin, it was shown to protect neurons against various types of injury. Wogonin inhibited the glutamate-or NMDA-induced excitotoxic neuronal damage in primary cultured rat cortical cells. It is well established that glutamate is extensively released during and after ischemia, and activation of various subtypes of its receptor causes direct and indirect Ca\(^{2+}\) influx in to the cell, resulting in neuronal damage. Thus, the capability of inhibiting excitotoxic neuronal damage may contribute, at least in part, to the neuroprotective effect of wogonin in either a transient global ischemia model or a permanent focal ischemia model as shown in this study.

Furthermore, wogonin was reported to inhibit the H\(_2\)O\(_2\)-induced damage in neuroblastoma cells. We previously expanded this observation in the primary cultured cortical cells. Wogonin inhibited oxidative neuronal injury induced by H\(_2\)O\(_2\), xanthine/xanthine oxidase, or glutathione depletion in the cultured cortical cells. It is believed that reactive oxygen or nitrogen radicals play crucial roles in neuronal death in many neurodegenerative diseases including cerebral ischemia. Therefore, the ability of wogonin to protect neurons against the oxidative damage in the cultured cells may also contribute, in some degree, to its neuroprotective effect in vivo. Based on these in vitro and in vivo findings, wogonin may exert neuroprotective effects through the actions on both neurons and microglia.

The root of *S. baikalensis*, Scutellariae radix, has been commonly used in traditional Oriental medicine for the treatment of various diseases including stroke. Its methanol extract has also been reported to inhibit microglial TNF-\(\alpha\) and NO production in vitro, and exhibit neuroprotective effects in vivo against transient global ischemia. Moreover, it was also demonstrated to reduce the ischemia/reperfusion-induced transient focal ischemic brain injury. It will be interesting to investigate whether it exhibits neuroprotective effect in the permanent focal ischemia model.

Attempts have been made to determine the active principle(s) responsible for the neuroprotective effect of *S. baikalensis*. Based on the findings in this study and previous reports, wogonin may be one of the critical components in...
the extract that mediate the neuroprotective effects. In addition to wogonin, baicalein and baicalin are the major constituents in *S. baicalensis*. Baicalein was also found to be neuroprotective in models of transient global ischemia in rats as well as in gerbils, although its effect was slightly lower than that of wogonin at the same dose. It was shown to be effective in the ischemia/reperfusion-induced transient focal ischemia model as well. These principles such as wogonin and baicalein and other component(s), if any, in the *S. baicalensis* extract may act together to exert neuroprotective effects.

Based on the results presented in this study, we demonstrated and confirmed the neuroprotective effect of wogonin using a permanent model of focal brain ischemia in rats. It reduced the permanent focal ischemic brain injury induced by pMCAO and improved behavioral deficits in rats. These findings, along with the previous findings, may provide strong pharmacological basis for the use of wogonin or *S. baicalensis* in the treatment of stroke.

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