Neuroprotective Effect of *Scutellaria baicalensis* Against MCAo Induced Focal Cerebral Ischemia

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ABSTRACT
Scutellaria baicalensis Georgi has been used for the treatment of various chronic inflammatory syndromes including respiratory disease, fever and gastric ulcer in traditional Eastern medicine and its major components; baicalin, baicalein and wogonin; were reported to have various biological effects. Previously, neuroprotective effect of *S. baicalensis* and its major flavonoids have been reported to have neuroprotective effect in in vitro. The aim of this study was to evaluate the neuroprotective effect of *S. baicalensis* in middle cerebral artery occlusion (MCAo) rat model. The rats were pretreated with *S. baicalensis* extract (100 mg/kg) administered after 0, 1 and 6 h of MCAo. The neuroprotective effect against acute ischemic stroke was evaluated by measuring the Infarct volume in both control and sample treated group. *S. baicalensis* (100 mg/kg) significantly reduced the infarct volume from 21.9 ± 5.95% in control group to the 10.8 ± 5.29% ($p<0.001$) in *S. baicalensis* treated group. Our study suggested that *S. baicalensis* has potential neuroprotective effect and these findings may be one of the alternative therapies for the management of stroke and other neurodegenerative diseases.

Key words: *Scutellaria baicalensis, Neuroprotection, Ischemia, Middle cerebral artery occlusion*

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INTRODUCTION
Cerebral ischemic stroke is a neurological disease where neuronal cell death is characterized by serial pathophysiological events, so called ischemic cascades, like energy failure, excitotoxicity, oxidative stress, inflammation and apoptosis. These all damaging factors are triggered by either decreased or blocked blood flow that leads to the human death and disability.[1,2] Two major approaches have been developed for the management of ischemic stroke. First approach is to establish reperfusion by dissolution of the clot with thrombolytic drugs and the second is to treat with neuroprotective agents to interfere with the biochemical cascade of events leading to cell death in the penumbra area.[3,4]

Due to the lack of effective and widely applicable pharmacological treatments for ischemic stroke, many people are generating their interests in traditional medicines, mainly of herbal origin.[5] Several natural products have been studied for their potential neuroprotective effects in past few decades.[6] Meanwhile, *Scutellaria baicalensis* Georgi has been generated great deal of attention for neuroprotection in animal model.

S. baicalensis is one of the popular medicinal plants in traditional Korean medicine. It is used for the treatment of high fever, jaundice, ulcer, inflammation and cancer. The root of *S. baicalensis* has been reported to contain essential oils, diterpenoids, amino acids and flavonoids.[7] The main bioactive flavonoids in *S. baicalensis* are baicalein, baicalin (baicalein-7-glucuronide), wogonin, wogonoside (wogonin-7-glucuronide), oroxylin A and oroxylin A-7-glucuronide.[8] *S. baicalensis* and its flavones have been studied for their various pharmacological activities, including anti-inflammatory, antibacterial, antiviral, antitumor, antioxidative, neuroprotective and anticonvulsant activities.[9-15]

In the previous study, our group reported the neuroprotective effects of *S. baicalensis* extract and wogonin in 4-vessel occlusion (4-VO) model, a widely used model to represent the transient global ischemia.[16,17] The in vitro neuroprotective effect of *S. baicalensis* has also been reported. However, the neuroprotective effect of the *S. baicalensis* in MCAo induced focal ischemic model has not been elucidated yet. In the present study, we aimed to evaluate the neuroprotective effect of *S. baicalensis* root extract in MCAo induced rat model of cerebral ischemia.

MATERIALS AND METHODS
Plant Material and extraction procedures
Dried root of *S. baicalensis* was purchased from Kyungdong Oriental drug store, Seoul, Korea. It was identified by Dr. H. Choi, Department of Herbal Pharmacology, College of Oriental Medicine, Kyung Hee University, Seoul, Korea. Voucher specimen, HP21001, has been deposited at the Herbarium of the College of Oriental Medicine.

Figure 1: *S. baicalensis* plant, rhizome and dried root pieces

1.5 kg of dried raw materials was extracted with 85% methanol (MeOH) by sonication. The mixture was filtered and the residue re-extracted twice. The filtrate was evaporated under vacuum to give 500 g methanol extract, which was stored at -20°C until use.

Animals and Drug Treatment
All animal handling procedures were performed in compliance with the animal welfare guidelines issued by the Korean National Institute of Health (KNIH) and the Korean Academy of Medical Sciences. Male Sprague Dawley Rat (SLC, Japan), weighing 300±5 g were used in the experiment. They were housed under controlled conditions (22 ± 2°C;

lighting 07:00-19:00 with constant humidity). Before the experiment, food was withheld overnight but water was made freely available.

Focal ischemia-reperfusion was produced by a modification of the monofilament method described by Zea *et al.,*[18] Rats were anesthetized with isoflurane in N_2O/O_2 and were allowed to breathe spontaneously during the operat ion period. A ventral neck incision was made and the left external carotid artery (ECA) and internal carotid artery (ICA) were exposed and carefully isolated. Twentyfive mm of nylon suture, tip rounded by silicon, was inserted into ECA and advanced into the ICA with minimal stretching and pulling out the vessels. The suture advanced intracranially to occlude the origin of middle cerebral artery (MCA). The MCA was occluded for a period of 60 minutes, after which rats were re-anesthetized and the suture was retracted to the bifurcation of the ICA and CCA. S. baicalensis (100mg/kg) was administered intraperitonially at 0, 1 and 6 h after the MCAo.

Histology and photomicrographing

48 h after the MCAo, rats were decapitated and then the brain removed. Using a brain matrix, 6 sections each 2 mm thick were cut and incubated in 2% TTC (triphryenyltetrazolium chrloride) for 30 min at 37°C. After incubation, slices were transferred to 4% formalin. Image analysis was done by taking the photographs which were further studied to determine the infarct area of both hemispheres for each slice by using Optimas 6.5 software system. Infarct volume (mm^3) was derived by the integration of the area measurements. The areas of infarct size in left hemisphere were calculated for each brain slice by subtracting the area of normal tissue in the ipsilateral hemisphere from the total area of the contralateral hemisphere. The infarct area for each slice was multiplied by slice thickness, and the results for each slice were summed to obtain the total corrected infarct volume for each animal.

Statistical Analysis

All data were presented as mean ± S.E.M. Student's t-test was used to make statistical comparisons between different treatment groups. $p<0.05$ were considered to be statistically significant

RESULTS

Infarct Areas in TTC Stained Brain Section

MCAo rats exhibited higher infarct area in the cortex and striatum than that of sham- operated rats. Infarct area was significantly reduced when S. baicalensis (100mg/kg) extract was administered by intraperitoneal injection 0, 1 and 6 hr after MCAo (Figure 2). Administration of S. baicalensis exhibited to significant reduce infarct volume in MCAo rats (Figure 3). The calculated rates between vehicle- and S. baicalensis treated groups were 21.9 ±5.95 % (n=7) and 10.8±5.29 % (n=8).

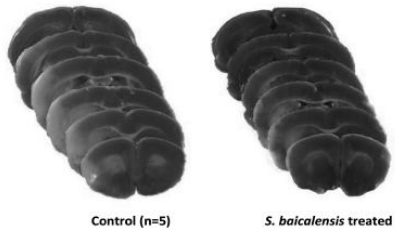

Figure 2: TTC stained brain section of control and *S. baicalensis* treated rat after MCAo. Each column demonstrates the series of rat brain coronal sections. Dark pink area indicates normal area and white area indicate infarct area. Thickness of each section is 2 mm.

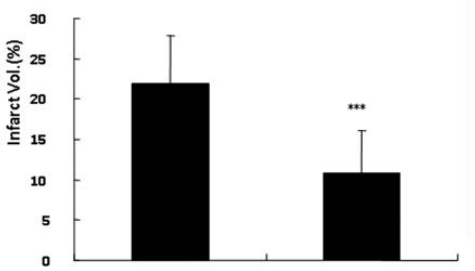

Figure 3: The effect of *S. baicalensis* on MCAo rat models after 48 h reperfusion. Following 60 min of ischemia, CO is control. S.B. is *S. baicalensis* (100 mg/kg) was i.p. administrated into the animals at 0, 1and 6 h after occlusion. Numbers in parenthesis are numbers of animals. Values are means ± SEM (*** $p<0.001$).

DISCUSSION

Over the last decade, the rat has become the predominant species for models of focal cerebral ischemia. Up to now, different techniques simulating human cerebral ischemia have been established in rats. Among the endovascular techniques for middle cerebral artery occlusion (MCAo), the suture occlusion technique in rats is the most frequently used method.[19-21] In this model, a monofilament is implemented into the internal carotid artery (ICA) until it blocks blood flow to the middle cerebral artery (MCA). This technique provides reproducible MCA territory infarctions and allows reperfusion by releasing the suture. Permanent MCAO with the suture technique, however, has one disadvantage: insertion of the suture occludes the entire course of the ICA, including the hypothalamic artery. This approach leads to hypothalamic infarctions that cause severe hyperthermia with effects on infarct growth, confounding treatment effects.[22,23]

In the present study, we determined the neuroprotective effect of *S. baicalensis* methanol extract on focal cerebral ischemia in rat. The infarct area in sample treated group was significantly reduced as compare to that of the control group. This study demonstrates that *S. baicalensis* infused i.p. starting 0, 1 and 6 h after induction of focal brain ischemia significantly reduces postmortem infarct size in rats and shows a trend toward delayed ischemia lesion volume shrinkage in vivo.

It has been reported that the cellular damage that occurs during cerebral ischemia and reperfusion is at least partly due to oxidative and inflammatory stress.[24] In neurodegenerative diseases including ischemia, reactive oxygen species have a deleterious effect on neuron survival. Therefore, antioxidants have been highlighted in neuroprotective drugs development.[25,26] Flavonoids isolated from *S. baicalensis* are reported to have the free radical-scavenging capacity and protective activity against injury.[13] *S. baicalensis* and its flavonoids are recognized as most powerful antioxidant. The potent neuroprotective activity of baicalein might be related to its antioxidative activity and its possible structure-activity relationship. Brain ischemia initiates a complex cascade of metabolic events, several of which involve the generation of nitrogen and oxygen free radicals.

These free radicals and related reactive chemical species mediate much of damage that occurs after transient brain ischemia, and in the penumbral region of infarcts caused by permanent ischemia.[27] Two important pathophysiological mechanisms involved during ischemic stroke are oxidative stress and inflammation. Brain tissue is not well equipped with antioxidant defenses, so reactive oxygen species and other free radicals/oxidants, released by inflammatory cells,

threaten tissue viability in the vicinity of the ischemic core.[28] We also determined the anti-inflammatory and antioxidant effect of *S. baicalensis* methanol extract (Data are not shown) and found that S. baicalensis possess potent antioxidant and anti-inflammatory effect.

In conclusion *S. baicalensis* possess the significant neuroprotective effect against MCAo induced focal cerebral ischemia in in vivo. The possible mechanism of neuroprotection might be due to the antioxidant and anti-inflammatory activity of *S. baicalensis*.

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