Journal of Ethnopharmacology xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

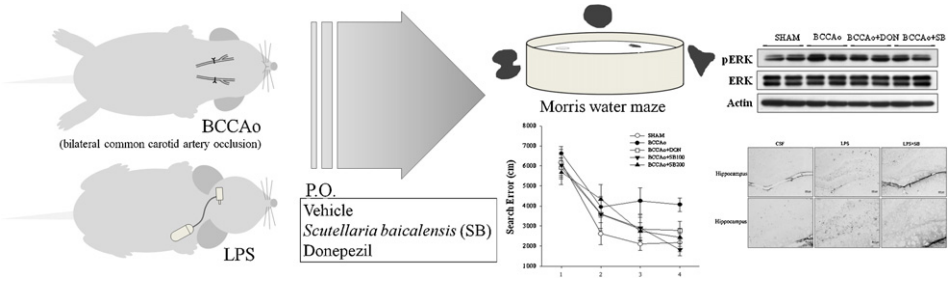
journal homepage: www.elsevier.com/locate/jethpharm

Effects of *Scutellaria baicalensis* on chronic cerebral hypoperfusion-induced memory impairments and chronic lipopolysaccharide infusion-induced memory impairments

Yoo Kyeong Hwang[a], Ma Jinhua[a], Bo-Ryoung Choi[a], Chun-Ai Cui[b], Won Kyung Jeon[c], Hocheol Kim[d], Hahn Young Kim[e], Seol-Heui Han[e], Jung-Soo Han[a,e,*]

[a] *Department of Biological Sciences, Konkuk University, Seoul 143-701, Republic of Korea*
[b] *Department of Anatomy, Yanbian University School of Basic Medical Science, Yanbian 133-000, China*
[c] *Traditional Korean Medicine Converging Research Division, Korea Institute of Oriental Medicine, Daejeon 305-811, Republic of Korea*
[d] *Department of Herbal Pharmacology, College of Oriental Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea*
[e] *Department of Neurology, Konkuk University Hospital, Center for Geriatric Neuroscience Research, IBST, Konkuk University, Seoul 143-729, Republic of Korea*

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 February 2011
Received in revised form 8 June 2011
Accepted 13 June 2011
Available online xxx

Keywords:
Scutellaria baicalensis
Alzheimer's disease
Cerebral hypoperfusion
Lipopolysaccharide
Spatial memory
MAPK
Microglia
Vascular dementia

ABSTRACT

Ethnopharmacological relevance: Extracts of the roots of *Scutellaria baicalensis* Georgi (Labiatae) have been widely used to relieve fever related to bacterial infection and inflammatory diseases in traditional Korean medicine and have been reported to be effective in brain diseases.

These experiments were conducted to examine the effects of oral administration of *Scutellaria baicalensis* extracts on the rescue of memory impairments induced by chronic cerebral hypoperfusion or chronic lipopolysaccharide (LPS) infusion. In addition, the underlying mechanisms of these effects were investigated.

Materials and methods: In the first experiment, chronic cerebral hypoperfusion was induced in male Wister rats by bilateral common carotid artery occlusion (BCCAo). Daily administration of *Scutellaria baicalensis* extracts was started on 20 day after BCCAo and given for 40 days. A Morris water maze was then used to evaluate the status of the hippocampal-dependent spatial learning and hippocampal mitogen-activated protein kinase (MAPK) signaling was examined in control rats, rats with chronic cerebral hypoperfusion, and rats with chronic cerebral hypoperfusion that was administered *Scutellaria baicalensis*. In the second experiment, hippocampal microglial activation was induced by chronic infusions of LPS into the fourth ventricle of Fisher-344 rat brains. Daily administration of *Scutellaria baicalensis* extracts was started on 7 day after the surgery of LPS infusion and given for 32 days. Spatial memory and hippocampal microglial activation was then examined in control rats with an artificial cerebrospinal fluid infusion, rats with chronic LPS infusion, and rats with chronic LPS infusion that were administered *Scutellaria baicalensis*.

* Corresponding author at: Department of Biological Sciences, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea. Tel.: +82 2 450 3293; fax: +82 2 3436 5432.
E-mail address: jshan06@konkuk.ac.kr (J.-S. Han).

0378-8741/$ – see front matter
doi:10.1016/j.jep.2011.06.025

Results: Rats that received chronic cerebral hypoperfusion or chronic LPS infusion showed spatial memory impairments relative to their control rats; however, these symptoms were reduced by daily administration of *Scutellaria baicalensis*. Administration of *Scutellaria baicalensis* mitigated alterations of hippocampal MAPK signaling by chronic cerebral infusion and microglial activation by chronic LPS infusion.
Conclusions: These results indicate that *Scutellaria baicalensis* may possess therapeutic potential for the prevention of Alzheimer's disease and vascular dementia.

1. Introduction

Neuroinflammatory processes clearly occur in pathologically vulnerable regions of brain in the several neurodegenerative disorders including Alzheimer's disease (AD) and vascular dementia (VaD) (Tomimoto et al., 1997; Akiyama et al., 2000; Ravaglia et al., 2007). It has also been reported that non-steroidal anti-inflammatory drugs (NDAIDs) prevent cognitive decline in individuals diagnosed with AD or elderly people (Tabet and Feldmand, 2003; Hayden et al., 2007). However, long-term treatment of NSAID produces gastrointestinal pain and occasional liver and kidney toxicity (Warner et al., 1999; Stichtenoth and Frolich, 2000; Graupera et al., 2003). These side effects have stimulated the development of new NDAIDs that are safe during long-term treatments (Wenk et al., 2002; Jin et al., 2008). Another alternative is the search for herbal medicines that have traditionally been used to treat diseases related to inflammation.

One traditional Korean herbal medicine is the root extract of *Scutellaria baicalensis* Georgi (*S. baicalensis*). This extract has been used to treat bacterial infection of the respiratory system and inflammatory diseases. Recently, the extract of *Scutellaria baicalensis* was shown to have neuroprotective effects on hippocampus lesions induced by ibotenic acid and global cerebral ischemia, as well as spinal cord injury (Kim et al., 2001; Heo et al., 2009; Yune et al., 2009). The three major flavonoids of *Scutellaria baicalensis*, baicalein, baicalin, and wogonin, have also been evaluated *in vivo* and *in vitro* (Lee et al., 2003; Suk et al., 2003; Kim et al., 2006).

Chronic cerebral hypoperfusion is associated with the development of VaD (Dubois and Hebert, 2001). Animal models that use permanent occlusion of the bilateral common carotid artery (BCCAo) have been used to understand the role of chronic cerebral hypoperfusion in the development of cognitive decline in VaD and to further develop its treatment (Tsuchiya et al., 1992; Wakita et al., 1998; Tomimoto et al., 2003). Animals with BCCAo show reduced cerebral blood flow and memory impairments similar to those that occur in human aging, AD, and VaD (Ohta et al., 1997; Otori et al., 2003). MAPKs play a crucial role in signal transduction and in the development of AD, which are activated by phosphorylation in response to mitogenic stimuli (Haddad, 2004). Therefore, the first experiment of the present study was conducted to examine the effects of the extract of *Scutellaria baicalensis* on hippocampal dependent memory impairments and alterations of hippocampal mitogen-activated protein kinases (MAPKs) signaling induced by BCCAo.

In addition, the second experiment of present study assessed the effects of the extract of *Scutellaria baicalensis* on hippocampal neuroinflammatory responses and hippocampal memory impairments induced by chronic LPS infusions. Specifically, the chronic infusion of lipopolysaccharide (LPS) into the fourth ventricle of young Wistar rats produced inflammatory responses in the hippocampus, such as activated microglia, which are consistent with early reports (Hauss-Wegrzyniak et al., 1998). In addition, the chronic LPS infusions were found to produce memory impairments (Hauss-Wegrzyniak et al., 1998; Jin et al., 2008). In this experiment, administration of the extract of *Scutellaria baicalensis* attenuated the neuroinflammatory responses and lessened the spatial memory impairments induced by chronic LPS infusions.

2. Materials and methods

2.1. Animals

Thirty-five male Wistar rats were used in a chronic BCCAo experiment (12 weeks old; Charles River Co., Gapeung, South Korea). The rats were housed in a vivarium at Konkuk University for two weeks at the beginning of the experiment under controlled temperature ($22 \pm 1\,^{\circ}C$) and humidity ($50 \pm 10\%$) on a 12 h light/dark cycle (lights on at 08:00 h). In addition, thirty male Fisher-344 rats obtained from SLC Inc. (Hamamatsu, Shizuoka, Japan), were used in a chronic LPS experiment. The Fisher-344 rats were 12 weeks old at the beginning of the experiment and resided in a vivarium at Konkuk University for at least two months prior to the experiment. Food and water were ad libitum provided to all animals throughout the experiment. The Institutional Animal Care and Use Committee of Konkuk University approved all protocols described in the report. All surgical procedures and behavioral testing took place during the light phase.

2.2. Surgery

2.2.1. BCCAo

The Wistar rats were anesthetized using a mixture of 5% isoflurane and oxygen and anesthesia was maintained with 3% isoflurane during the surgical procedure. A midline incision was made to expose the bilateral common carotid arteries, which were then tightly double ligated with silk sutures. In addition, control animals were subjected to a sham-operation in which they underwent the same procedure as the treatment animals, without bilateral common carotid artery occlusion. The rectal temperature was maintained at $37.0 \pm 0.5\,^{\circ}C$ with a heating pad throughout the surgical procedure. During hypoperfusion, about 5% of the animals showed neurological symptoms like seizure with squatting, and these animals died within one week after surgery. In addition, rats that lost their weight 80% than before the surgery were excluded from further experiments.

2.2.2. Chronic LPS injection

The Fisher-344 rats were anesthetized with isoflurane and then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA) fitted with an isoflurane gas anesthesia system and an incisor bar set 3.3 mm below the ear bars. LPS ($n = 20$) or artificial cerebrospinal fluid (aCSF, $n = 10$) was chronically infused through a cannula implanted in the fourth ventricle of the brain and was attached to an osmotic minipump (Alzet, Palo Alto, CA, USA, model 2004). Each minipump was prepared to inject either the vehicle artificial cerebrospinal fluid (aCSF) or 250 ηg LPS/h (prepared in aCSF). The composition of the aCSF (in mmol/L) was 140 NaCl; 3.0 KCl; 2.5 $CaCl_2$; 1.2 Na_2HPO_4, pH 7.4. The details were described elsewhere (Rosi et al., 2004).

2.3. Drug and administration

Scutellaria baicalensis was obtained from Kyung-Dong Herbal Market in Seoul, Korea and authenticated and deposited in the herbarium of Kyung Hee University in October 2007. Dried roots

Please cite this article in press as: Hwang, Y.K., et al., Effects of *Scutellaria baicalensis* on chronic cerebral hypoperfusion-induced memory impairments and chronic lipopolysaccharide infusion-induced memory impairments. J. Ethnopharmacol. (2011), doi:10.1016/j.jep.2011.06.025

of *Scutellaria baicalensis* (1.0 kg) were extracted by water using traditional oriental method. Briefly, *Scutellaria baicalensis* (50 g) was extracted with 2 L of boiling water for 2 h, filtered, and then lyophilized (yield: 34.8%). The powdered extract (*Scutellaria baicalensis*; pH 4.72) was subsequently dissolved in saline and filtered through a 0.22-μm syringe filter (Yoon et al., 2009). The extract of *Scutellaria baicalensis* contains several flavonoids, such as wogonin, baicalin, and oroxylin (Heo et al., 2009). HPLC analysis was conducted to confirm the contents of the extracts of *Scutellaria baicalensis* used for the present experiment. Analysis was performed in a Waters 600 pump, a Waters 717 autosampler, and a Waters 996 Photodiode Array Detector was used for all analyses. Chromatographic separations were carried out on a Sunfire™ C18 ODS column (250 mm × 4.6 mm id, 5 μm) with a Sunfire™ C18 Guard column (250 mm × 4.6 mm id, 5 μm). The mobile phases were composed of acetonitrile (A) and 0.5% phosphoric acid (B) using the following gradient program: 0–60 min, 5–50% A; 60–61 min, 50–70% A; 61–80 min, 70–70% A; 80–81 min, 70–5% A. The absorbance was measured at 276 nm for detection of baicalin, wogonoside. Chromatography was performed at room temperature at a flow rate of 1.0 mL/min and the injection volume was 10 μL. The contents of baicalin and wogonoside were 19.83 ± 0.91% and 5.47 ± 0.36%, respectively.

The rats used for the BCCAo experiment were segregated into five groups (six rats per group): a sham-operated group (oral administration of the drug vehicle); BCCAo group (oral administration of the drug vehicle); three BCCAo groups that received daily oral administration of drugs (*Scutellaria baicalensis* 100 mg/kg, *Scutellaria baicalensis* 200 mg/kg, donepezil (10 mg/kg). These dosages were determined based on the studies conducted with *Scutellaria baicalensis* (Shang et al., 2005; Heo et al., 2009). Donepezil is used to treat dementia associated with AD and VaD. Vehicle/drug treatment was started on day 20 after BCCAo surgery and continued until the end of the experiment (see Fig. 1A). During drug administration, one rat was lost in each drug treatment group.

The following three groups were used in the chronic LPS experiment ($n = 10$): (1) aCSF-infused rats that received daily oral administration of the drug vehicle; (2) LPS-infused rats that received daily oral administration of the drug vehicle; (3) LPS-infused rats that received daily oral administration of *Scutellaria baicalensis* (100 mg/kg). Vehicle/drug treatment was started on day seven after the LPS surgery and continued until the end of the experiment (see Fig. 1B). During drug administration, one rat was lost in each group that received chronic LPS infusion. The drug vehicle for *Scutellaria baicalensis* was deionized water.

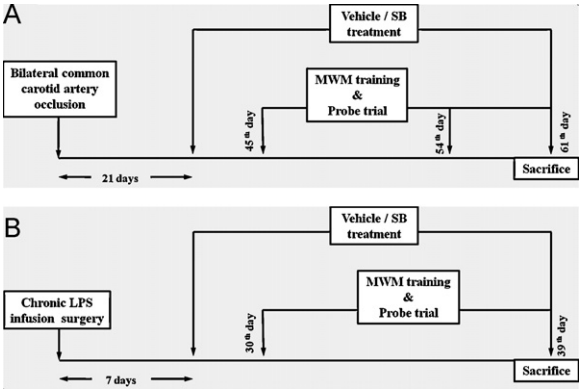


Fig. 1. Experimental design for chronic BCCAo (A) and chronic LPS (B).

2.4. Behavioral assessment

2.4.1. Apparatus

The maze was a round tank, 1.83 m in diameter and 58 cm deep, and filled to a depth of 35.5 cm with tepid (26 ± 1 °C) water made opaque by the addition of white paint (tempera). A moveable circular platform, 12 cm in diameter, was located 2 cm below the surface of the water. The maze was surrounded by white curtains on which black cloth visual stimuli of various shapes and sizes were placed. A camera located above the center of the maze relayed images to a videocassette recorder and an HVS Image Analysis Computer System. Data from the water maze trials were analyzed using software provided by HVS (Hampton, United Kingdom).

2.4.2. Training procedure of spatial memory task for chronic BCCAo rats

In a standardized procedure that required the use of distal cues in a maze environment, the rats were trained to learn the position of a camouflaged escape platform (Gallagher et al., 1993). Briefly, every session contained five trials across two days and the total training was composed of four sessions. During each training trial, the location of the platform remained constant and the rats swam for 90 s or until they found the platform. Across the trials, the starting location varied among four equidistant points around the perimeter of the apparatus. A probe trial was conducted 30 min after the second session and the fourth session to assess the development of spatial bias in the maze; thus, the entire training procedure included two probe trials for each rat. During these probe trials, the rats swam with the platform retracted to the bottom of the pool for 30 s, at which time the platform was raised to its normal position for completion of the trial. The behavioral assessment was started on day 45 after surgery.

2.4.3. Training procedure for spatial memory task for chronic LPS rats

The rats were trained using the same training apparatus and procedure except that there were no the probe trials. The minipump for the chronic infusion is expected to deliver 0.25 μL/h for 32 days, which estimated by information provided by Alzet. Therefore, the behavioral assessment was started on day 30 day after chronic LPS infusion.

2.5. Western blot analysis

Proteins for the analysis of MAPKs and pMAPKs were extracted in the following manner. Individual tissue samples were weighed and then homogenized in five volumes of ice-cold buffer containing 20 mM Tris at pH 7.5, 5% glycerol, 1.5 mM EDTA, 40 mM KCl, 0.5 mM dithiothreitol, and protease inhibitors (No. 539131, Calbiochem). The homogenates were then centrifuged at 20,800 × *g* for 30 min at 4 °C, after which the supernatant was removed from each sample, and an aliquot was taken for determination of the total protein concentration using Bradford Reagent. The proteins were then separated by SDS-PAGE and transferred to a PVDF membrane, which was subsequently incubated with a primary antibody (Ab) against ERK (1:5000, Cell Signaling), JNK (1:5000, Cell Signaling), p38 (1:1000, Cell Signaling), pERK (1:3000, Cell Signaling), pJNK (1:3000, Cell Signaling), and pp38 (1:1000, Cell Signaling). Following primary incubation, blots were incubated with horseradish peroxidase (HRP)-conjugated secondary Ab (1:2500, Amersham Biosciences) and then visualized using an ECL system, after which they were developed using Hyperfilm (Amersham). The relative expression levels of MAPKs, and pMAPKs were determined by densitometry and normalization to β-actin (1:5000, Sigma), an invariant cytoskeletal protein.

Please cite this article in press as: Hwang, Y.K., et al., Effects of *Scutellaria baicalensis* on chronic cerebral hypoperfusion-induced memory impairments and chronic lipopolysaccharide infusion-induced memory impairments. J. Ethnopharmacol. (2011), doi:10.1016/j.jep.2011.06.025

2.6. Immunohistology

Following the behavioral experiments, the rats were euthanized by an overdose of ketamine HCl (30 mg/kg) and xylazine (2.5 mg/kg), after which they were intracardially perfused with 4% paraformaldehyde in a 0.1 M phosphate buffer (pH 7.4, PPB). The brain was then removed and cut coronal at Lambda (rostral to caudal) to partition the portion of the brain containing fourth ventricle (verified by the position of the cannula tip) from the brain portion containing hippocampus/cortex. Following fixation, the brains were removed, postfixed in PPB (2 h), cryoprotected in PBS containing 20% sucrose (24 h), frozen on powdered dry ice, and then sectioned (coronal plane: 40 um) using a microtome. Monoclonal antibody OX-6 (1:300, BD Bioscience), which is directed against the Class II major histocompatibility complex antigens, was used to visualize the activated microglial cells (Hauss-Wegrzyniak et al., 2002). For OX-6 immunoreactivity, endogenous peroxidases were blocked by 30 min of incubation in 3% H_2O_2/10% MeOH in PBS. The sections were then incubated for 1 h (RT) in PBS with 0.3% Triton-X containing 10% serum, followed by 18 h incubation (4 °C) in the same solution with the addition of OX-6 primary antibody. Next, the sections were incubated for 1 h in the appropriate biotinylated secondary (RT; 1: 200), and then for 1 h in ExtrAvidin peroxidase conjugate (RT; 1: 1000), after which they were reacted using a Vector SG substrate kit for peroxidase (Vector Laboratories). Finally, the sections were mounted onto Superfrost++ slides, dehydrated through ascending concentrations of alcohol, defatted in xylene, and coverslipped with Permount.

2.7. Statistical analysis

One-way ANOVA and one-way repeated ANOVA were conducted to assess the effects of *Scutellaria baicalensis* on the changes in pMAPKs and the impairment of spatial memory induced by chronic BCCAo or chronic LPS infusion. Post hoc analyses (Turkey HSD or *T*-test) were subsequently conducted to determine the effects of the *baicalensis* treatment. *p* Values less than 0.05 were considered significant, unless otherwise specified.

3. Results

3.1. Scutellaria baicalensis reduced chronic BCCAo-induced spatial memory impairments

In the Morris water maze task, search errors that are described in detail elsewhere (Gallagher et al., 1993) were used to assess the performance accuracy of spatial learning in the water maze. During each trial, the distance of the rats from the escape platform was sampled 10 times per second, and these values were averaged in 1-s bins. The cumulative search error was then calculated as the summed 1-s averages of this proximity measure corrected for the particular start location on each trial. One-way repeated ANOVA showed that the between group effects (sham-operated control, BCCAo with vehicle, BCCAo with drug treatments) were significant ($F_{(4,22)} = 4.18$, $p = 0.012$), as were the training effects (session) ($F_{(3,66)} = 99.77$, $p = 0.000$). The interaction effects between the group and training session were not significant. As shown in Fig. 2A, the sham-operated control rats quickly became proficient at locating the submerged platform during the training sessions; however, the BCCAo rats did not show much improvement over the course of training when compared with the control rats. This was confirmed by post hoc analysis, which revealed significant differences between the sham-operated control rats and the BCCAo rats ($p = 0.006$). In addition, the BCCAo rats that were administered *Scutellaria baicalensis* (100 mg/kg) showed significantly better performances than the BCCAo rats ($p = 0.05$). The BCCAo rats with *Scutellaria baicalensis* (200 mg/kg) or donezepil (10 mg/kg) showed no significant differences in performance when compared to the BCCAo rats.

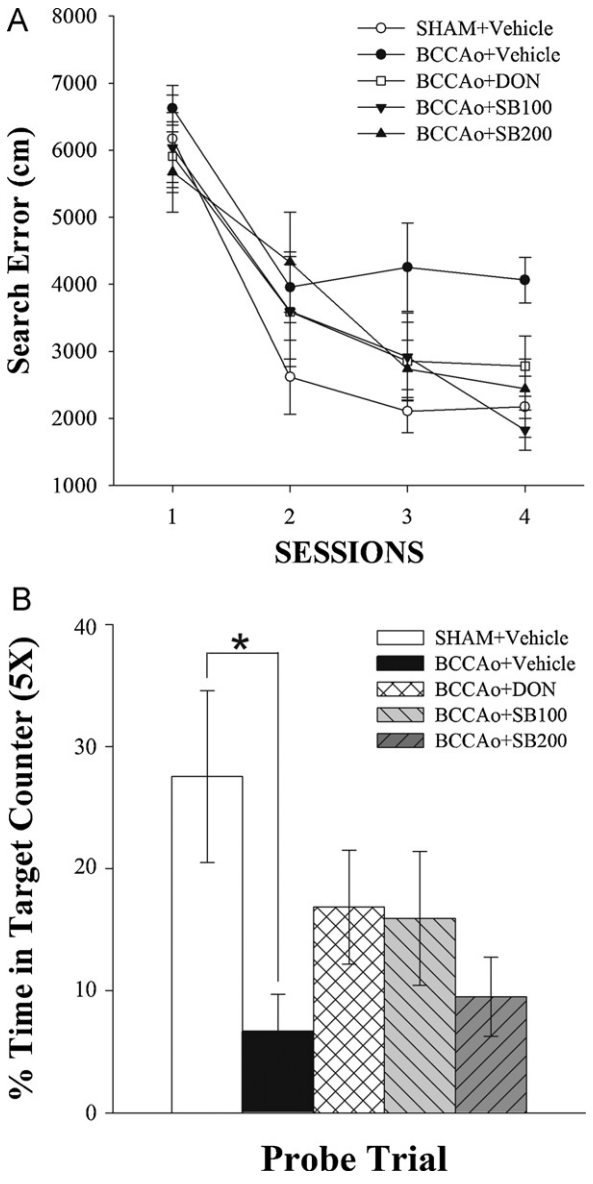


Fig. 2. *Scutellaria baicalensis* rescues spatial memory impairment induced by chronic BCCAo. (A) Search error for finding the hidden platform in the spatial learning task. Sham-operated control rats (SHAM + vehicle) became proficient at locating the submerged platform during the training trials. The BCCAo rats (BCCAo + vehicle) did not show much improvement over the course of the training, whereas the *Scutellaria baicalensis* (100 mg/kg, BCCAo + SB100) treatment significantly ameliorated this learning deficit induced by chronic BCCAo. (B) The percentage of time spent in the target annulus (5× of the platform size). Sham-operated control rats showed the spatial bias. However, the BCCAo rats treated with *Scutellaria baicalensis* did not show statistically significantly reduced effects in the probe trial when compared with the BCCAo rats. SHAM + vehicle: sham-operated control; BCCAo + vehicle: BCCAo rats; BCCAo + DON: BCCAo rats with donezepil (10 mg/kg); BCCAo + SB100: BCCAo rats with *Scutellaria baicalensis* (100 mg/kg); BCCAo + SB200: BCCAo rats with *Scutellaria baicalensis* (200 mg/kg).

In addition, although no apparent differences in performance in the first probe trial were observed, there were apparent differences in performance in the second probe trial, as assessed by the percentage of time spent in the target annulus (5× of the platform size) during the 30 s probe. The one-way ANOVAs on the

Please cite this article in press as: Hwang, Y.K., et al., Effects of *Scutellaria baicalensis* on chronic cerebral hypoperfusion-induced memory impairments and chronic lipopolysaccharide infusion-induced memory impairments. J. Ethnopharmacol. (2011), doi:10.1016/j.jep.2011.06.025

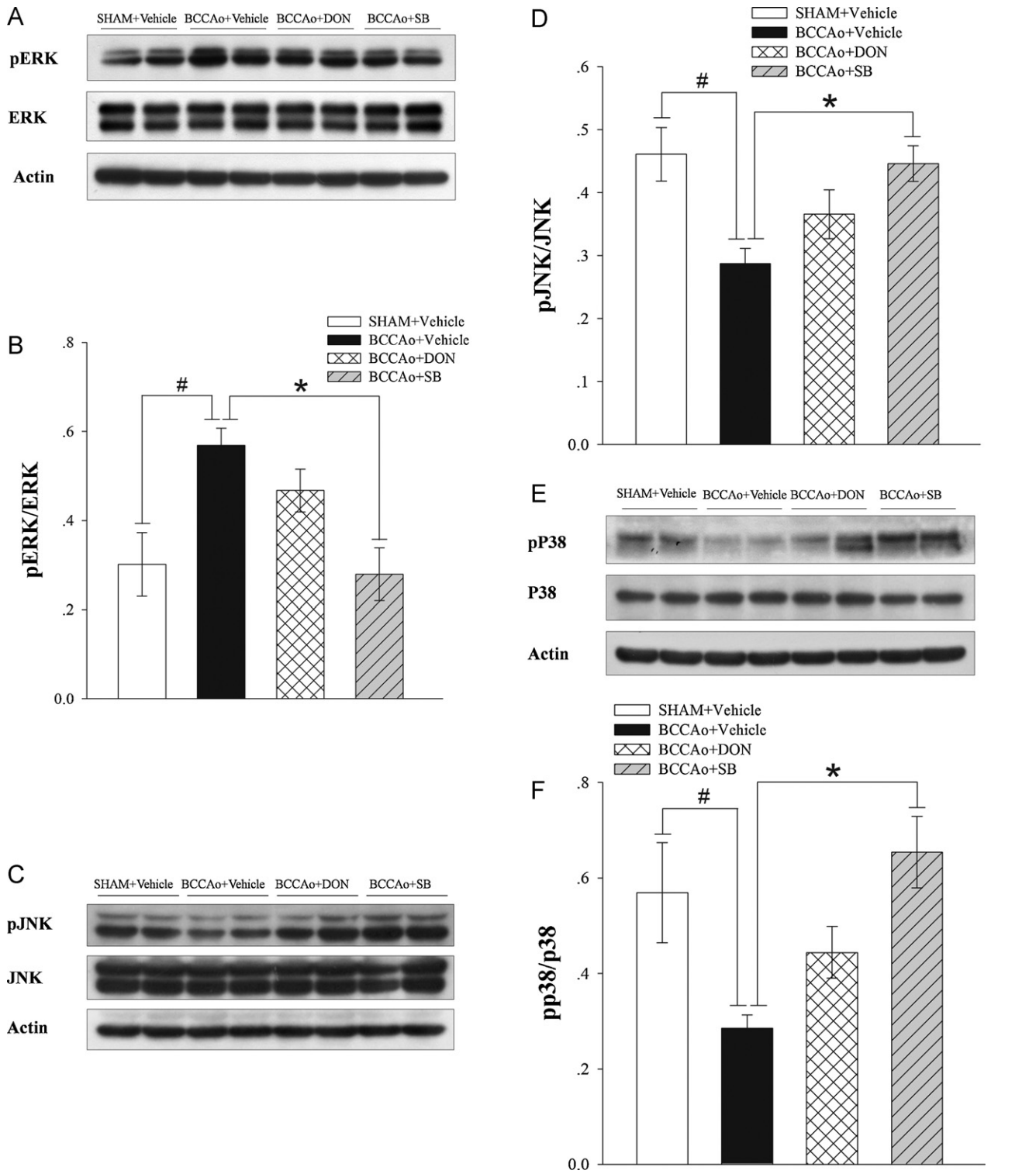


Fig. 3. *Scutellaria baicalensis* normalized alterations of the hippocampal MAPKs phosphorylation induced by chronic BCCAo. (A) Representative western blot of pERK (top), ERK (middle), and actin (bottom). (B) Chronic BCCAo increased pERK levels in the hippocampus compared with the control (#), and *Scutellaria baicalensis* suppressed increased hippocampal pERK levels induced by chronic BCCAo (*). (C) Representative western blot of pJNK (top), JNK (middle), and actin (bottom). (D) Chronic BCCAo decreased hippocampal pJNK levels compared with the control (#), and the hippocampal pJNK abundances of the chronic BCCAo rats with *Scutellaria baicalensis* were nearly identical to those of control (*). (E) Representative western blot of pp38 (top), p38 (middle), and actin (bottom). (F) Hippocampal pp38 levels were decreased in the brains of chronic BCCAo rats relative to the control (#), and decreased hippocampal pp38 levels by chronic BCCAo were normalized by *Scutellaria baicalensis* treatment (*). SHAM + vehicle: sham-operated control; BCCAo + vehicle: BCCAo rats; BCCAo + DON: BCCAo rats with donezepil; BCCAo + SB100: BCCAo rats with *Scutellaria baicalensis*.

second probe trial showed that the between group effects were significant ($F_{(4,22)} = 2.791$, $p = 0.05$). Post hoc analysis revealed that the sham-operated control rats were found to have the spatial bias when compared with the BCCAo rats that were treated with vehicle ($p = 0.02$) (Fig. 2B). However, BCCAo rats subjected to drug treatments did not show statistically significant ameliorative effects during the probe trials when compared with BCCAo rats that were treated with vehicle ($p > 0.09$).

Please cite this article in press as: Hwang, Y.K., et al., Effects of *Scutellaria baicalensis* on chronic cerebral hypoperfusion-induced memory impairments and chronic lipopolysaccharide infusion-induced memory impairments. J. Ethnopharmacol. (2011), doi:10.1016/j.jep.2011.06.025

3.2. *Scutellaria baicalensis* normalized BCCAo-induced alteration of MAPKs in the hippocampus.

MAPK activation plays a major role in synaptic plasticity and hippocampus-dependent memory (Sweatt, 2001). The present experiment was conducted to determine if BCCAo induced alterations of MAPKs in the hippocampus, which are required for spatial learning, and if *Scutellaria baicalensis* treatment normalized its alterations. Specifically, we examined three subfamilies of MAPKs: extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38. The hippocampal pMAPK levels were measured in the brains of the three rats in four groups (sham-operated control, BCCAo + vehicle, BCCAo + *Scutellaria baicalensis* (100 mg/kg), BCCAo + donezepil), by western blot. One-way ANOVA analysis of the pERK showed that the between group effects were significant ($F_{(3,22)} = 6.19$, $p = 0.018$). According to the post hoc analysis, the hippocampal pERK levels in the BCCAo rats were strongly upregulated when compared with the sham-operated control (Fig. 3A and B; $p = 0.038$). However, these increases induced by BCCAo were attenuated by treatment with *Scutellaria baicalensis* ($p = 0.026$). One-way ANOVA analysis of the pJNK showed that the between group effects were significant ($F_{(3,22)} = 5.49$, $p = 0.024$). Moreover, post hoc analyses revealed that the hippocampal pJNK levels in the BCCAo rats were significantly decreased when compared with the sham-operated controls (Fig. 3C and D; $p < 0.029$). These decreases induced by BCCAo were normalized by treatment with *Scutellaria baicalensis* ($p < 0.045$). One-way ANOVA of the pp38 showed that the between group effects were significant ($F_{(3,22)} = 5.06$, $p = 0.030$). Post hoc analyses revealed that the hippocampal pp38 levels in BCCAo rats were significantly lower than those in the sham-operated control (Fig. 3E and F; $p < 0.050$), which was consistent with results of pJNK. These decreases induced by BCCAo were attenuated by treatment with *Scutellaria baicalensis* ($p < 0.023$).

3.3. *Scutellaria baicalensis* reduced LPS-induced spatial memory impairments

Search error was used to assess the performance accuracy in the water maze. The overall repeated ANOVAs showed that the between group effects (aCSF, LPS, or LPS with *Scutellaria baicalensis*) were significant ($F_{(2,25)} = 4.95$, $p = 0.015$), as were the training effects (sessions) ($F_{(3,75)} = 81.82$, $p = 0.000$). There were no interaction effects between group and training session ($F_{(6,75)} = 0.57$, ns). As shown in Fig. 4, the aCSF-infused rats rapidly became proficient at locating the submerged platform during the training trials. However, the LPS-infused rats did not show much improvement over the course of the training when compared with the control aCSF-infused rats ($p = .027$), while *Scutellaria baicalensis* treatment ameliorated this learning deficit induced by chronic LPS infusions ($p = 0.006$).

3.4. *Scutellaria baicalensis* reduced LPS-induced microglia activation in the hippocampus

The locations of the cannula tips were verified while using a microtome to cut the portion of the brain containing the fourth ventricle. All cannula tips were located in the fourth ventricle. Immunostaining for OX-6 revealed numerous activated microglias distributed in the hippocampus of the LPS-infused rats (Fig. 5A). The activated microglia had a characteristic bushy morphology with increased cell body size and contracted and ramified processes (Wenk, 2003). To quantify the effects of the *Scutellaria baicalensis* treatments, the number of OX-6 positive microglia within the hippocampi from every rat were counted in drawings of identical sections. ANOVA revealed significant group effects in the hippocampus ($F_{(2,25)} = 112.04$, $p = 0.000$). Post hoc analyses of the group effects revealed that the number of activated microglia in the hippocampi of LPS-infused rats was significantly higher than that of the aCSF-infused rats and LPS-infused rats treated with *Scutellaria baicalensis* ($p < 0.001$); however, the number of activated microglia in LPS-infused rats with *Scutellaria baicalensis* was significantly greater than in aCSF-infused rats ($p < 0.001$) (Fig. 5B).

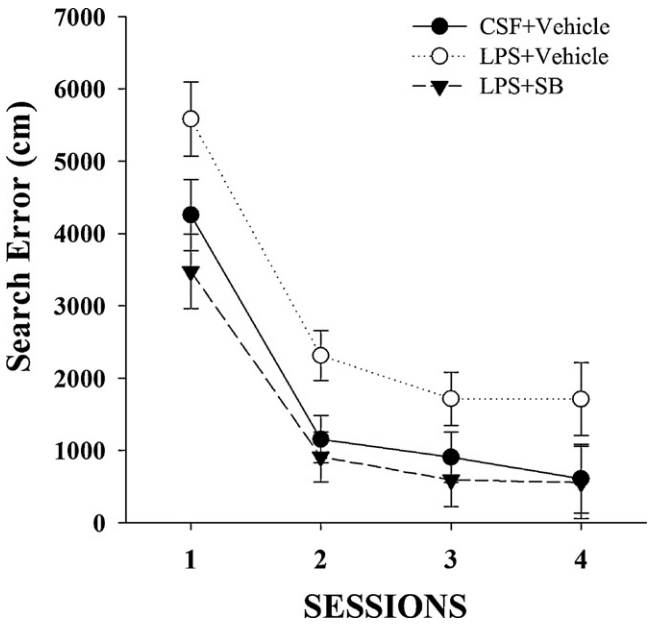


Fig. 4. *Scutellaria baicalensis* reduced LPS-induced spatial memory impairments. The control rats became proficient at locating the submerged platform during the training trials. The LPS-infused rats did not show much improvement over the course of the training when compared with the control aCSF-infused rats, while the *Scutellaria baicalensis* treatment ameliorated this learning deficit by chronic LPS infusions. CSF + vehicle: aCSF-infused rat; LPS + vehicle: LPS-infused rat; LPS + SB: LPS-infused rat with *Scutellaria baicalensis*.

4. Discussion

The therapeutic effects of *Scutellaria baicalensis* have been demonstrated in a number of studies using animal models for AD and VaD, which are rats with permanent global ischemia (Kim et al., 2001; Shang et al., 2005) and rats with lesions of entorhinal cortex induced by ibotenic acid (Heo et al., 2009), respectively. The present study showed that the root extract of *Scutellaria baicalensis* is beneficial for amelioration of cognitive deficits induced by chronic cerebral hypoperfusion or chronic neuroinflammation. In addition, the results of the present study revealed that signaling of hippocampal MAPKs was altered in rats with chronic BCCAo, and that this alteration was normalized by the treatment of *Scutellaria baicalensis.*

The root extract of *Scutellaria baicalensis* is a traditional herbal medicine in Korea and China that is widely used to treat inflammatory diseases and bacterial infection of the respiratory and gastrointestinal tract (Liu et al., 2007; Heo et al., 2009). *Scutellaria baicalensis* contains several flavonoids, including wogonin, baicalin, and oroxylin A. The effectiveness of *Scutellaria baicalensis* has been demonstrated by animal models for AD and VaD, and its molecular mechanism of their action has been investigated by cell culture studies, with its extract or single components. For example, treatment of baicalein ameliorated memory impairments induced by chronic BCCAo or β-amyloid peptide (25–35) infusion, indicating that there is a relationship between improved behavioral performances by baicalein and ERK signaling (Wang et al., 2004; Liu et al., 2007; Park et al., 2010). Oroxylin A also ameliorated memory

Please cite this article in press as: Hwang, Y.K., et al., Effects of *Scutellaria baicalensis* on chronic cerebral hypoperfusion-induced memory impairments and chronic lipopolysaccharide infusion-induced memory impairments. J. Ethnopharmacol. (2011), doi:10.1016/j.jep.2011.06.025

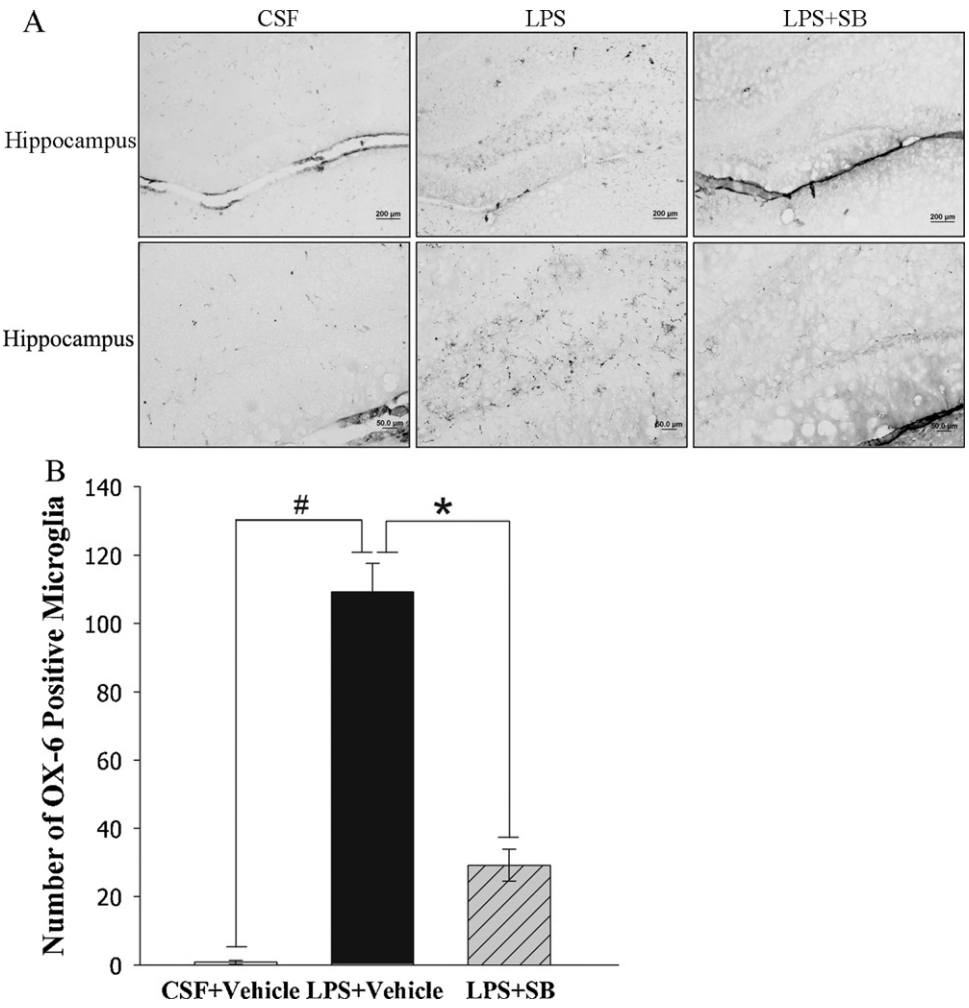


Fig. 5. Immunohistology for activated microglia using the OX-6 antibody in the hippocampus of Fisher-344 rats. A. Representative hippocampal photomicrograph of aCSF control rats (left), chronic LPS-infused rats (middle), and chronic LPS-infused rat treated with daily administrations of *Scutellaria baicalensis* (right). B. The number of activated microglia in the hippocampi of Fisher-344 rats. The number of activated microglia in the hippocampus of the LPS-infused rats was significantly greater than in aCSF-infused rats (#). The *Scutellaria baicalensis* treatments significantly reduced the number of activated microglia in the hippocampi of the LPS-infused rats (*). CSF + vehicle: aCSF-infused rat; LPS + vehicle: LPS-infused rat; LPS + SB: LPS-infused rat treated with daily administrations of *Scutellaria baicalensis*.

impairments induced by chronic BCCAo or scopolamine injection in mice (Kim et al., 2006; Kim et al., 2007). In contrast, to the best of our knowledge, no studies have been reported the ameliorating effects of wogonin on memory impairments using an animal model. Nevertheless, there have been some reports of wogonin having neuroprotective effects in cerebral ischemic insult, and these effects have been shown to be mediated by its anti-inflammatory properties via suppression of microglial activation through the inhibition of NF-kB activity (Cho and Lee, 2004; Piao et al., 2008).

Thus, the effectiveness of its extract or its single components of *Scutellaria baicalensis* for AD and VaD has been reported using various animal models. However, no studies have been conducted to compare the efficacy of the extract of *Scutellaria baicalensis* with that of individual components or to determine if there were any differences in efficacy among single components with respect to behavioral improvements and alterations of signature markers of inflammation/oxidative stress. Accordingly, it is premature to adopt a single component of *Scutellaria baicalensis* with the highest efficacy for development of a new drug for the treatment of AD and VaD. Moreover, based on the complexity of treatment *in vivo*, a combinatory approach through the use of an extract would be preferable to the use of single components (Pratico, 2008). Hence, the results of the present experiment conducted using the extracts of *Scutellaria baicalensis* are useful for investigating its therapeutic effects.

Recent lines of research support the possibility that a superfamily of MAPKs may be a key factor in the regulation of Tau and deposits of amyloid precursor protein (beta-APP), eventually leading to AD, and that alterations in signaling pathways of MAPKs may underlie the mechanism of synaptic pathology in AD and VaD (Hashimoto and Masliah, 2003; Haddad, 2004). Furthermore, treatments that induced alterations in MAPKs signaling resulted in impairments in hippocampal long-term potentiation (LTP) and spatial learning deficits (Atkins et al., 1998; Selcher et al., 1999). Therefore, the present experiment compared the levels of phosphorylated MAPKs in the hippocampi of chronic BCCAo rats to those of control rats. Hippocampal levels of pERK were increased and hippocampal levels of pJNK and pp38 were decreased, in chronic BCCAo rats relative to control rats. More importantly, these alterations in MAPK signaling induced by BCCAo were normalized by treatment of *Scutellaria baicalensis*. These findings suggest that chronic BCCAo results in cognitive deficits due to impairments of synaptic plasticity. Accordingly, it is necessary to determine if hippocampal LTP is impaired in rats with chronic BCCAo.

Chronic infusion of LPS into the 4th ventricle of Fisher-344 rat brains produces many of the inflammatory symptoms,

Please cite this article in press as: Hwang, Y.K., et al., Effects of *Scutellaria baicalensis* on chronic cerebral hypoperfusion-induced memory impairments and chronic lipopolysaccharide infusion-induced memory impairments. J. Ethnopharmacol. (2011), doi:10.1016/j.jep.2011.06.025

pathological changes, and memory impairments associated with AD (Hauss-Wegrzyniak et al., 1998). Specifically, chronic LPS infusion increases the number and density of OX-6-positive reactive microglia, which are the immune competent cells of the central nervous system (Hauss-Wegrzyniak et al., 2000) and it increases the number and density of astrocytes (Hauss-Wegrzyniak et al., 1998). In the present study, an animal model of inflammation was used to assess the anti-inflammatory effects of *Scutellaria baicalensis*. The results showed that chronic LPS infusions in Fisher-344 rats activated inflammatory responses in the hippocampus, such as activated microglia. Chronic LPS infusions have been shown to impair spatial memory in a Morris water maze (Hauss-Wegrzyniak et al., 1999), which have been reported in our previous studies (Cui et al., 2008; Jin et al., 2008). Here, we demonstrate that oral administration of *Scutellaria baicalensis* attenuates the neuroinflammatory responses induced by chronic LPS infusions and also reduces the spatial memory impairments induced by the chronic LPS infusions.

5. Conclusion

Using the chronic BCCAo animal model for VaD and the LPS infusion animal model for AD, the present study examined the *in vivo* efficacies of *Scutellaria baicalensis*. Daily treatments with *Scutellaria baicalensis* normalized MAPKs signaling in the hippocampus of chronic BCCAo. These treatments also reduced the spatial memory impairments that were induced by chronic BCCAo. Furthermore, daily treatments with *Scutellaria baicalensis* attenuated activated inflammatory responses and spatial memory impairments induced by chronic LPS infusions. Taken together, these results may lead to the development of novel anti-dementia treatments for AD, VaD, and other CNS diseases.

Acknowledgments

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0008867) to Hahn Young Kim and a grant (kiom-2010-2) from the Inter-Institutional Collaboration Research Program provided by the Korea Research Council of Fundamental Science & Technology (KRCF), Korea.

References

Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G.M., Cooper, N.R., Eikelenboom, P., Emmerling, M., Fiebich, B.L., Finch, C.E., Frautschy, S., Griffin, W.S., Hampel, H., Hull, M., Landreth, G., Lue, L., Mrak, R., Mackenzie, I.R., McGeer, P.L., O'Banion, M.K., Pachter, J., Pasinetti, G., Plata-Salaman, C., Rogers, J., Rydel, R., Shen, Y., Streit, W., Strohmeyer, R., Tooyoma, I., Van Muiswinkel, F.L., Veerhuis, R., Walker, D., Webster, S., Wegrzyniak, B., Wenk, G., Wyss-Coray, T., 2000. Inflammation and Alzheimer's disease. Neurobiology of Aging 21, 383–421.

Atkins, C.M., Selcher, J.C., Petraitis, J.J., Trzaskos, J.M., Sweatt, J.D., 1998. The MAPK cascade is required for mammalian associative learning. Nature Neuroscience 1, 602–609.

Cho, J., Lee, H.K., 2004. Wogonin inhibits ischemic brain injury in a rat model of permanent middle cerebral artery occlusion. Biological and Pharmaceutical Bulletin 27, 1561–1564.

Cui, C.A., Jin, D.Q., Hwang, Y.K., Lee, I.S., Hwang, J.K., Ha, I., Han, J.S., 2008. Macelignan attenuates LPS-induced inflammation and reduces LPS-induced spatial learning impairments in rats. Neuroscience Letters 448, 110–114.

Dubois, M.F., Hebert, R., 2001. The incidence of vascular dementia in Canada: a comparison with Europe and East Asia. Neuroepidemiology 20, 179–187.

Gallagher, M., Burwell, R., Burchinal, M., 1993. Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. Behavioral Neuroscience 107, 618–626.

Graupera, M., Garcia-Pagan, J.C., Abraldes, J.G., Peralta, C., Bragulat, M., Corominola, H., Bosch, J., Rodes, J., 2003. Cyclooxygenase-derived products modulate the increased intrahepatic resistance of cirrhotic rat livers. Hepatology 37, 172–181.

Haddad, J.J., 2004. Mitogen-activated protein kinases and the evolution of Alzheimer's: a revolutionary neurogenetic axis for therapeutic intervention? Progress in Neurobiology 73, 359–377.

Hashimoto, M., Masliah, E., 2003. Cycles of aberrant synaptic sprouting and neurodegeneration in Alzheimer's and dementia with Lewy bodies. Neurochemical Research 28, 1743–1756.

Hauss-Wegrzyniak, B., Dobrzanski, P., Stoehr, J.D., Wenk, G.L., 1998. Chronic neuroinflammation in rats reproduces components of the neurobiology of Alzheimer's disease. Brain Research 780, 294–303.

Hauss-Wegrzyniak, B., Lynch, M.A., Vraniak, P.D., Wenk, G.L., 2002. Chronic brain inflammation results in cell loss in the entorhinal cortex and impaired LTP in perforant path-granule cell synapses. Experimental Neurology 176, 336–341.

Hauss-Wegrzyniak, B., Vannucchi, M.G., Wenk, G.L., 2000. Behavioral and ultrastructural changes induced by chronic neuroinflammation in young rats. Brain Research 859, 157–166.

Hauss-Wegrzyniak, B., Vraniak, P., Wenk, G.L., 1999. The effects of a novel NSAID on chronic neuroinflammation are age dependent. Neurobiology of Aging 20, 305–313.

Hayden, K.M., Zandi, P.P., Khachaturian, A.S., Szekely, C.A., Fotuhi, M., Norton, M.C., Tschanz, J.T., Pieper, C.F., Corcoran, C., Lyketsos, C.G., Breitner, J.C., Welsh-Bohmer, K.A., 2007. Does NSAID use modify cognitive trajectories in the elderly? The Cache County study. Neurology 69, 275–282.

Heo, H., Shin, Y., Cho, W., Choi, Y., Kim, H., Kwon, Y.K., 2009. Memory improvement in ibotenic acid induced model rats by extracts of *Scutellaria baicalensis*. Journal of Ethnopharmacology 122, 20–27.

Jin, D.Q., Sung, J.Y., Hwang, Y.K., Kwon, K.J., Han, S.H., Min, S.S., Han, J.S., 2008. Dexibuprofen (S(+)-isomer ibuprofen) reduces microglial activation and impairments of spatial working memory induced by chronic lipopolysaccharide infusion. Pharmacology, Biochemistry, and Behavior 89, 404–411.

Kim, D.H., Jeon, S.J., Son, K.H., Jung, J.W., Lee, S., Yoon, B.H., Choi, J.W., Cheong, J.H., Ko, K.H., Ryu, J.H., 2006. Effect of the flavonoid, oroxylin A, on transient cerebral hypoperfusion-induced memory impairment in mice. Pharmacology, Biochemistry, and Behavior 85, 658–668.

Kim, D.H., Jeon, S.J., Son, K.H., Jung, J.W., Lee, S., Yoon, B.H., Lee, J.J., Cho, Y.W., Cheong, J.H., Ko, K.H., Ryu, J.H., 2007. The ameliorating effect of oroxylin A on scopolamine-induced memory impairment in mice. Neurobiology of Learning and Memory 87, 536–546.

Kim, Y.O., Leem, K., Park, J., Lee, P., Ahn, D.K., Lee, B.C., Park, H.K., Suk, K., Kim, S.Y., Kim, H., 2001. Cytoprotective effect of *Scutellaria baicalensis* in CA1 hippocampal neurons of rats after global cerebral ischemia. Journal of Ethnopharmacology 77, 183–188.

Lee, H., Kim, Y.O., Kim, H., Kim, S.Y., Noh, H.S., Kang, S.S., Cho, G.J., Choi, W.S., Suk, K., 2003. Flavonoid wogonin from medicinal herb is neuroprotective by inhibiting inflammatory activation of microglia. FASEB Journal 17, 1943–1944.

Liu, C., Wu, J., Gu, J., Xiong, Z., Wang, F., Wang, J., Wang, W., Chen, J., 2007. Baicalein improves cognitive deficits induced by chronic cerebral hypoperfusion in rats. Pharmacology, Biochemistry, and Behavior 86, 423–430.

Ohta, H., Nishikawa, H., Kimura, H., Anayama, H., Miyamoto, M., 1997. Chronic cerebral hypoperfusion by permanent internal carotid ligation produces learning impairment without brain damage in rats. Neuroscience 79, 1039–1050.

Otori, T., Katsumata, T., Muramatsu, H., Kashiwagi, F., Katayama, Y., Terashi, A., 2003. Long-term measurement of cerebral blood flow and metabolism in a rat chronic hypoperfusion model. Clinical and Experimental Pharmacology and Physiology 30, 266–272.

Park, S.J., Kim, D.H., Kim, J.M., Shin, C.Y., Cheong, J.H., Ko, K.H., Ryu, J.H., 2010. Mismatch between changes in baicalein-induced memory-related biochemical parameters and behavioral consequences in mouse. Brain Research 1355, 141–150.

Piao, H.Z., Choi, I.Y., Park, J.S., Kim, H.S., Cheong, J.H., Son, K.H., Jeon, S.J., Ko, K.H., Kim, W.K., 2008. Wogonin inhibits microglial cell migration via suppression of nuclear factor-kappa B activity. International Immunopharmacology 8, 1658–1662.

Pratico, D., 2008. Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: lights and shadows. Annals of the New York Academy of Sciences 1147, 70–78.

Ravaglia, G., Forti, P., Maioli, F., Chiappelli, M., Montesi, F., Tumini, E., Mariani, E., Licastro, F., Patterson, C., 2007. Blood inflammatory markers and risk of dementia: The Conselice study of brain aging. Neurobiology of Aging 28, 1810–1820.

Rosi, S., Ramirez-Amaya, V., Hauss-Wegrzyniak, B., Wenk, G.L., 2004. Chronic brain inflammation leads to a decline in hippocampal NMDA-R1 receptors. Journal of Neuroinflammation 1, 12.

Selcher, J.C., Atkins, C.M., Trzaskos, J.M., Paylor, R., Sweatt, J.D., 1999. A necessity for MAP kinase activation in mammalian spatial learning. Learning and Memory 6, 478–490.

Shang, Y., Cheng, J., Qi, J., Miao, H., 2005. Scutellaria flavonoid reduced memory dysfunction and neuronal injury caused by permanent global ischemia in rats. Pharmacology, Biochemistry, and Behavior 82, 67–73.

Stichtenoth, D.O., Frolich, J.C., 2000. COX-2 and the kidneys. Current Pharmaceutical Design 6, 1737–1753.

Suk, K., Lee, H., Kang, S.S., Cho, G.J., Choi, W.S., 2003. Flavonoid baicalein attenuates activation-induced cell death of brain microglia. Journal of Pharmacology and Experimental Therapeutics 305, 638–645.

Sweatt, J.D., 2001. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. Journal of Neurochemistry 76, 1–10.

Tabet, N., Feldmand, H., 2003. Ibuprofen for Alzheimer's disease. Cochrane Database of Systematic Reviews, CD004031.

Tomimoto, H., Akiguchi, I., Wakita, H., Suenaga, T., Nakamura, S., Kimura, J., 1997. Regressive changes of astroglia in white matter lesions in cerebrovas-

Please cite this article in press as: Hwang, Y.K., et al., Effects of *Scutellaria baicalensis* on chronic cerebral hypoperfusion-induced memory impairments and chronic lipopolysaccharide infusion-induced memory impairments. J. Ethnopharmacol. (2011), doi:10.1016/j.jep.2011.06.025

cular disease and Alzheimer's disease patients. Acta Neuropathology 94, 146–152.
Tomimoto, H., Ihara, M., Wakita, H., Ohtani, R., Lin, J.X., Akiguchi, I., Kinoshita, M., Shibasaki, H., 2003. Chronic cerebral hypoperfusion induces white matter lesions and loss of oligodendroglia with DNA fragmentation in the rat. Acta Neuropathology 106, 527–534.
Tsuchiya, M., Sako, K., Yura, S., Yonemasu, Y., 1992. Cerebral blood flow and histopathological changes following permanent bilateral carotid artery ligation in Wistar rats. Experimental Brain Research 89, 87–92.
Wakita, H., Tomimoto, H., Akiguchi, I., Kimura, J., 1998. Dose-dependent, protective effect of FK506 against white matter changes in the rat brain after chronic cerebral ischemia. Brain Research 792, 105–113.
Wang, S.Y., Wang, H.H., Chi, C.W., Chen, C.F., Liao, J.F., 2004. Effects of baicalein on beta-amyloid peptide-(25–35)-induced amnesia in mice. European Journal of Pharmacology 506, 55–61.
Warner, T.D., Giuliano, F., Vojnovic, I., Bukasa, A., Mitchell, J.A., Vane, J.R., 1999. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. Proceedings of the National Academy of Sciences of the United States of America 96, 7563–7568.
Wenk, G.L., 2003. Neuropathologic changes in Alzheimer's disease. Journal of clinical Psychiatry 9, 7–10.
Wenk, G.L., Rosi, S., McGann, K., Hauss-Wegrzyniak, B., 2002. A nitric oxide-donating flurbiprofen derivative reduces neuroinflammation without interacting with galantamine in the rat. European Journal of Pharmacology 453, 319–324.
Yoon, S.B., Lee, Y.J., Park, S.K., Kim, H.C., Bae, H., Kim, H.M., Ko, S.G., Choi, H.Y., Oh, M.S., Park, W., 2009. Anti-inflammatory effects of *Scutellaria baicalensis* water extract on LPS-activated RAW 264.7 macrophages. Journal of Ethnopharmacology 125, 286–290.
Yune, T.Y., Lee, J.Y., Cui, C.M., Kim, H.C., Oh, T.H., 2009. Neuroprotective effect of *Scutellaria baicalensis* on spinal cord injury in rats. Journal of Neurochemistry 110, 1276–1287.

Please cite this article in press as: Hwang, Y.K., et al., Effects of *Scutellaria baicalensis* on chronic cerebral hypoperfusion-induced memory impairments and chronic lipopolysaccharide infusion-induced memory impairments. J. Ethnopharmacol. (2011), doi:10.1016/j.jep.2011.06.025