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Neuroprotective effects of a traditional herbal prescription on transient cerebral global ischemia in gerbils

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ABSTRACT

Aim of the study: Kyung-Ok-Ko (KOK), a traditional herbal prescription composed of *Rehmannia glutinosa* var. *purpurae*, *Panax ginseng*, *Poria cocos*, *Lycium chinense*, *Aquillaria agallocha* and honey, has been used to treat age-related symptoms, such as amnesia or dementia, and has been shown to ameliorate scopolamine-induced memory impairment in mice. However, the effects of KOK on transient cerebral global ischemia-induced brain damage are unclear.

Materials and methods: Transient cerebral global ischemia was induced by occluding the bilateral common carotid artery for 5 min followed by reperfusion for 7 days. KOK (0.25, 0.5, 1, or 2 g/kg) was administered orally immediately after reperfusion and once a day over the next 7 days. Y-maze or novel object recognition tasks were to analyze learning and memory capabilities at 4 or 5 days after reperfusion, respectively. Histochemistry and immunohistochemistry were used for evaluation of the effect of KOK on neuronal degeneration.

Results: Histochemical studies showed that KOK increased the number of viable cells detected by Nissl staining and decreased the number of degenerated neuronal cells detected by Fluoro-Jade B staining in the hippocampal CA1 region. In the immunohistochemical study, the sub-chronic KOK administration attenuated the ischemia-induced activation of microglia and astrocytes and the increase of cytokine IL-1β ($P<0.05$). In addition, KOK administration significantly attenuated the ischemia-induced cognitive impairments observed in the Y-maze and novel object recognition tasks ($P<0.05$).

Conclusion: These findings suggest that the neuroprotective effects of KOK may be mediated by its anti-inflammatory activities, resulting in the attenuation of memory impairment.

1. Introduction

Cerebral ischemic injury resulting from either focal or global circulatory arrests in the brain, is one of the major causes of death and disability in adults (Pinkston et al., 2009). Global cerebral ischemia is a clinical outcome occurring as a consequence of cardiac arrest, reversible severe hypotension or other situations that deprive the brain of oxygen and glucose (Nedergaard and Diemer, 1988; White et al., 1993). Multiple factors, including excitotoxicity, oxidative stress, and inflammatory cytokines (tumor necrosis factor-α, interleukin (IL)-1β or IL-6), are responsible for hippocampal neuronal damage (Endoh et al., 1994; Kasparova et al., 2005; Pinkston et al., 2009; Ikonomidou and Kaindl, 2010a,b, 2011), especially the pyramidal neurons of the CA1 region in the hippocampus (Kirino, 2000). Among these factors, the inflammatory response is a delayed process and could be a potential target for the treatment of brain ischemia (Yrjanheikki et al., 1999; Tuttolomondo et al., 2009). In line with inflammation in ischemic brain, glial cells including microglia and astrocytes are also involved in neuronal degeneration (Petito et al., 1990; Denes et al., 2007). Based on these observations, many drugs have been tested for their abilities to delay neuronal death, including cyclooxygenase-2 inhibitors (Gackowski et al., 2008; Hamel et al., 2008), nuclear factor-κB inhibitors (Ridder and Schwaninger, 2009), inducible nitric oxide synthase inhibitors (Cai et al., 2008; Shin et al., 2010),

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and inhibitors against glial cell activation (Muramatsu et al., 2004; Lee et al., 2011). However, when selective anti-inflammatory or anti-excitatory agents were applied in ischemic brain damage, satisfactory outcomes were not obtained (Guo et al., 2009).

Kyung-Ok-Ko (KOK; Qiong-yu-gao in Chinese; Kei-gyoku-kou in Japanese) is a traditional herbal prescription that contains six ingredients: *Rehmannia glutinosa* var. *purpurae*, *Panax ginseng*, *Poria cocos*, *Lycium chinense*, *Aquillaria agallocha* and honey. It has been used for age-related symptoms, such as amnesia and dementia in East Asia (Hur, 1999). Owing to the traditional use of KOK, we observed that KOK ameliorated scopolamine-induced memory impairments in mice (Shin et al., 2009). Some studies on the medicinal properties of KOK have also focused on immunological activities (Lee et al., 2002), inflammation (Lee et al., 2008), or gastric ulcers (Whang et al., 1994). From these studies, we hypothesized that KOK may be effective on inflammation-related brain disorders which exhibit memory impairment. However, no attempts have been made to investigate whether KOK has neuroprotective or ameliorative effects against ischemic brain damage-induced memory impairment. In recent years, various tries to develop anti-ischemic drugs from natural products are being conducted (Gupta et al., 2010). Therefore, the purpose of this study was to investigate whether KOK has neuroprotective effects on transient cerebral global ischemia-induced neuronal damage and whether it ameliorates ischemia-induced learning and memory deficits.

2. Materials and methods

2.1. Animals

Mongolian gerbils (60–80 g) were purchased from the Orient Co., Ltd., a branch of Charles River Laboratories (Seoul, Korea). Animals were housed 4 per cage, allowed access to water and food ad libitum, and maintained under a constant temperature ($23 \pm 1\,^{\circ}C$) and humidity ($60 \pm 10\%$) under a 12-h light/dark cycle (light on 07:30–19:30 h). Animal treatment and maintenance were conducted in accordance with the Principle of Laboratory Animal Care (NIH Publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Kyung Hee University, Seoul, Korea. For the experiments, gerbils were divided into six groups [sham, $n = 5$; ischemia, $n = 5$; ischemia + KOK (0.25 g/kg), $n = 5$; ischemia + KOK (0.5 g/kg), $n = 6$; ischemia + KOK (1 g/kg), $n = 6$; ischemia + KOK (2 g/kg), $n = 6$; ischemia + MK-801, $n = 6$; a total of 39 animals were used].

2.2. Materials

The goat anti-ionized calcium-binding adaptor molecule-1 (Iba-1) antibody and rabbit anti-interleukin-1β (IL-1β) antibody were purchased from Abcam (Cambridge, UK). The mouse anti-glial fibrillary acidic protein (GFAP) antibody, cresyl violet acetate and Fluoro-Jade B (FJ-B) were purchased from Chemicon (Temecula, CA). Biotinylated secondary antibody and avidin–biotin-peroxidase complex (ABC) kits were purchased from Vector (Burlingame, CA). MK-801, bovine serum albumin (BSA), and 3,3′-diaminobenzidine tetrahydrochloride (DAB) were purchased from Sigma Chemical Co. (St. Louis, MO). Zoletil 50® was purchased from Virbac laboratory (Carros, France). All other materials were of the highest grade commercially available.

2.3. Sample preparation

Kyung-Ok-Ko (100 g) was prepared following method. Juice of root of *Rehmannia glutinosa* Liboschitz var. *purpurae* Makino (Scrophulariaceae) (32.0 g), powder of dried root of *Panax ginseng* C.A. Meyer (Araliaceae) (2.8 g), powder of cortex of *Poria cocos* Wolf (Polyporaceae) (8.0 g), powder of dried fruit of *Lycium chinense* Miller (Solanaceae) (0.9 g), powder of resin of *Aquillaria agallocha* Roxburgh (Thymelaeaceae) (0.1 g), honey (38.5 g), and simple syrup (17.7 g) were mixed and heated at 80 °C in the water bath for 72 h. Viscous extract was obtained and used for present study. KOK (Lot No., OV30) standardized with 5-hydroxymethyl furaldehyde (9.4%) for consistency of quality was donated by Kwang Dong Pharmaceutical Co. (Pyongtaek, Korea).

2.4. Surgical procedure and drug administration

Animals were anesthetized with isoflurane (2.5% for induction, 1% for maintenance) in a mixture of nitrous oxide and oxygen (70:30), and the duration of anesthesia was no longer than 5 min. Transient cerebral ischemia was induced by the bilateral common carotid artery occlusion (BCCAO) as following. After making a median incision in the neck skin, both common carotid arteries were exposed and occluded with aneurysm clips for 5 min. Body temperature was maintained at $37 \pm 0.5\,^{\circ}C$ with a heating pad throughout surgery (Biomed S.L., Alicante, Spain). Circulation was restored by removing the clips. Animals receiving the same surgical operation without clipping the carotid arteries served as sham-operated controls. Regional cerebral blood flow (rCBF) was monitored using laser Doppler flowmetry (LDF; Perimed, PF5010, JarFalla, Sweden). Cyanoacrylate adhesives were used to attach flexible probes (model 407, Perimed, Jarfalla, Sweden) to the intact skull ±3.5 mm of the bregma. The change in rCBF was measured for 1 min immediately after occlusion and expressed as a percentage of the baseline value. The gerbil which showed over 80% of rCBF reduction was used for further study. After reperfusion, the animals were placed in a warm incubator (32–33 °C).

KOK (0.25, 0.5, 1, or 2 g/kg, p.o.) was administered once a day for 7 days beginning immediately after reperfusion until designated time points (Fig. 1). A group intraperitoneally treated with MK-801

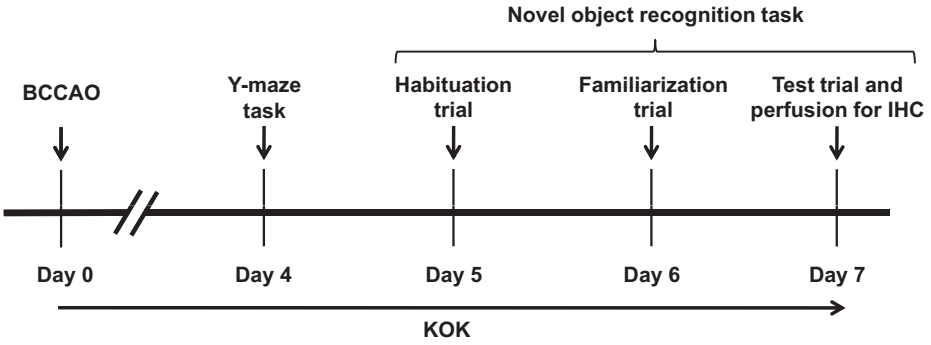

Fig. 1. Experimental procedures. BCCAO, bilateral common carotid artery occlusion; KOK, Kyung-Ok-Ko; IHC, immunohistochemistry.