Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Ameliorative effects of yokukansan on behavioral deficits in a gerbil model of global cerebral ischemia

Yanan Liu[a], Takehiro Nakamura[a,*], Tetsuhiko Toyoshima[a], Feng Lu[a], Kazunori Sumitani[b], Aya Shinomiya[c], Richad F. Keep[d], Tohru Yamamoto[a], Takashi Tamiya[c], Toshifumi Itano[a]

[a]Department of Neurobiology, Kagawa University Faculty of Medicine, Miki, Japan
[b]Department of Medical Education, Kagawa University Faculty of Medicine, Miki, Japan
[c]Department of Neurological Surgery, Kagawa University Faculty of Medicine, Miki, Japan
[d]Department of Neurosurgery, University of Michigan, Ann Arbor, MI, USA

ARTICLE INFO

Article history:
Accepted 14 November 2013
Available online 19 November 2013

Keywords:
Cerebral ischemia
Gerbil
Yokukansan
Kampo medicine
Behavior
Oxidative stress
Inflammation

ABSTRACT

The aim of this study was to investigate the neuroprotective effects of yokukansan, a traditional Kampo medicine, on the behavioral dysfunction induced by cerebral ischemia/reperfusion injury in gerbils. Gerbils were treated with yokukasan by oral gavage for 30 days, once per day, until the day before induction of ischemia, which was induced by occluding the bilateral common carotid artery for 5 min. The effects of yokukansan (50, 100 and 300 mg/kg) were examined by measuring neuronal damage and behavioral deficits (locomotor activity, 8-arm radial maze task). The anti-inflammatory and anti-oxidant properties of yokukansan were also examined. Administration of yokukansan at 300 mg/kg significantly reduced hippocampal neuronal death after brain ischemia, inhibited the ischemia-induced inflammatory response and DNA oxidative damage. Yokukansan also reduced ischemia-induced locomotor hyperactivity and improved memory impairment. These findings suggest that yokukansan can inhibit the inflammatory response, oxidative damage and subsequent neuronal death induced by cerebral ischemia/reperfusion injury, and also can contribute to improvement in neurological deficits following such injury.

1. Introduction

Ischemic stroke results from a transient or permanent reduction in cerebral blood flow. It causes neuronal death and associated behavioral deficits, including sensorimotor function, impaired spatial orientation, learning and memory impairment (Amano et al., 1993; Markgraf et al., 1992). A complex series of events including excitotoxicity, oxidative stress, blood–brain barrier dysfunction and postischemic inflammation ultimately lead to cell death of neurons and glia cells after cerebral ischemia (Diaz-Ruiz et al., 2008; Jin et al., 2010).

Yokukansan (YKS, Yi-gan San in Chinese) is a traditional Japanese medicine ("Kampo"), which is approved by the

*Correspondence to: Department of Neurobiology, Kagawa University Faculty of Medicine, 1750-1 Ikenobe, Miki, Kita, Kagawa 761-0793, Japan. Fax: +81 87 891 2208.
E-mail address: tanakamu@kms.ac.jp (T. Nakamura).

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.11.015

Ministry of Health, Labor and Welfare of Japan as a remedy of neurosis, insomnia, night crying and irritability in infants. Recently, YKS was reported to improve the behavioral and psychological symptoms of dementia (BPSD) such as aggression, anxiety and depression in patients with Alzheimer's disease, cerebrovascular dementia and other forms of senile dementia (Iwasaki et al., 2005a; Iwasaki et al., 2005b; Matsuda et al., 2013; Nagata et al., 2012). Although many studies indicate beneficial effects of YKS on BPSD, few studies have focused on the effect of YKS on the behavioral deficits induced by transient forebrain ischemia. The present study investigated the effect of YKS on behavioral deficits and hippocampal neuronal damage in a gerbil model of transient forebrain ischemia and examined the mechanisms underlying those effects.

2. Results

2.1. *Effect of YKS on ischemia-induced hippocampal neuronal death*

HE staining demonstrated a dose-dependent effect of YKS on hippocampal neuronal damage 72 h after global brain ischemia in gerbils (Fig. 1). The majority of pyramidal neurons within the hippocampal CA1 area of the ischemia group were shrunken and darkly stained with minimal cytoplasm compared to the sham group. In contrast, neurons within the same area in ischemia+300 mg/kg YKS treated animals were well preserved and appeared normal (Fig. 1A). As shown in Fig. 1B, ANOVA revealed a significant effect of YKS treatment ($F_{4,\ 24}=300.9$, $P<0.0001$). YKS 300 mg/kg treatment significantly reduced hippocampal neuronal loss. However, neither 50 mg/kg nor 100 mg/kg YKS provided neuroprotection (Fig. 1B).

The TUNEL method was used to detect DNA fragmentation. TUNEL-positive apoptotic cells, which contain dark brown stained nuclei were found in the ischemia group and both YKS 50 mg/kg and 100 mg/kg treatment groups (Fig. 2). No TUNEL-positive cells were detected in the hippocampal CA1 area in either the sham or YKS 300 mg/kg-treated groups (Fig. 2).

2.2. *Effect of YKS on ischemia-induced behavioral deficits*

YKS treatment also attenuated cerebral ischemia-induced behavioral deficits. As shown in Fig. 3A, ANOVA revealed a significant effect of YKS treatment ($F_{2,\ 11}=116.7$, $P<0.0001$). The locomotor activity counts in the ischemia group were significantly increased compared with the sham group 72 h after ischemia ($P<0.05$). The hyperlocomotion with ischemia was absent in animals treated with YKS (300 mg/kg) ($P<0.05$ vs. ischemia group; Fig. 3A).

Fig. 3B shows the number of errors recorded in the 8-arm radial maze task. Two-way repeated ANOVA revealed significant major effects of groups ($F_{2,\ 54}=6.03$; $P<0.01$) and trials ($F_{3,\ 54}=72.55$; $P<0.0001$). There was no interaction between the groups and trials ($F_{6,\ 54}=0.70$; $P>0.05$). In all groups, the number of errors tended to decrease with time. In untreated animals, the number of errors three days after ischemia was significantly increased compared to the sham group ($P<0.05$). Again, deficits were improved with YKS treatment with the number of errors being significantly decreased in the ischemia+YKS group compared to the ischemia group ($P<0.05$; Fig. 3B).

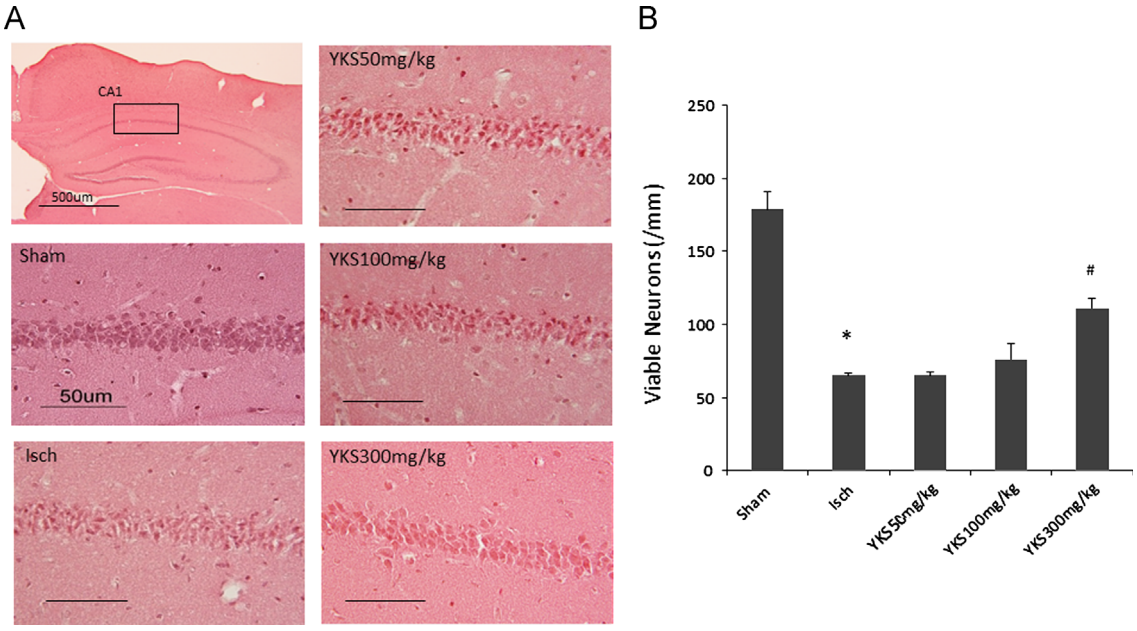

Fig. 1 – (A) HE staining in the hippocampal CA1 region, indicated in lower magnification (upper left; scale bar: 500 μm). Representative sections from the hippocampal CA1 region of sham (middle left), ischemia (Isch; lower left), yokukansan (YKS) treatment at 50 mg/kg (top right), 100 mg/kg (middle right) and 300 mg/kg (bottom right) groups on day 3 following transient brain ischemia (scale bar: 50 μm). (B) Viable neurons per millimeter of the hippocampal CA1 region. ($n=5$ per group; * $P<0.05$ vs. sham; # $P<0.05$ vs. ischemia).