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Integrative Medicine Research

journal homepage: www.imr-journal.com

Original Article

Yokukansan, a Kampo medicine, prevents the development of morphine tolerance through the inhibition of spinal glial cell activation in rats

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ARTICLE INFO

Article history:
Received 14 November 2015
Received in revised form
22 December 2015
Accepted 24 December 2015
Available online 31 December 2015

Keywords:
astrocyte
Kampo medicine
microglia
morphine tolerance
Yokukansan

ABSTRACT

Background: Animal models have shown that glial cells (microglia and astrocytes) in the spinal cord undergo activation following peripheral injury associated with chronic pain, suggesting the involvement of these cells in pain diseases. We have previously reported that Yokukansan (YKS), a Japanese traditional herbal (Kampo) medicine, is effective against chronic pain through the suppression of spinal glial cell activation. Morphine is a widely-used opioid analgesic for relieving severe pain, but its repeated administration leads to the development of antinociceptive tolerance. The development of morphine tolerance is also reported to be caused by spinal glial cells activation. In the present study, we investigated the inhibitory effects of YKS on the development of morphine tolerance and the activation of the spinal microglia and astrocytes using a rat model.

Methods: Male Wistar rats received a subcutaneous injection of morphine hydrochloride (10 mg/kg/d) for 7 days, and the withdrawal latency to thermal stimulation was measured daily using a hot plate test. Thereafter, the appearance of activated microglia and astrocyte in the spinal cord (L5) was examined by immunofluorescence staining. Ionized calcium binding adapter molecule-1 (Iba-1) staining was used to label microglia and glial fibrillary acidic protein (GFAP) staining was performed to label astrocytes. YKS was administered mixed with powdered rodent chow at a concentration of 3%.

Results: The preadministration of YKS (started 3 d before the morphine injection) prevented the development of morphine tolerance. The repeated administration of morphine increased Iba-1 and GFAP immune reactivities in the spinal cord; however, these activations were inhibited by the preadministration of YKS.

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http://dx.doi.org/10.1016/j.imr.2015.12.003

Conclusion: These results suggest that the preadministration of YKS attenuates the development of antinociceptive morphine tolerance, and the suppression of spinal glial cell activation may be one mechanism underlying this phenomenon.

1. Introduction

Glial cells are known to release various inflammatory cytokines and neurotrophic factors, such as adenosine triphosphate, interleukin (IL) -6 and -1β, tumor necrosis factor (TNF)-α, and nitric oxide, that lead to the regulation of neuronal functions and synaptic contacts.[1] Studies using animal models have reported that the glial cells (microglia and astrocytes) in the spinal cord undergo structural and functional modifications following peripheral injuries associated with chronic pain, suggesting the involvement of these cells in pain.[2,3] Therefore, these cells may potentially serve as targets for pain therapy.

Morphine is an opioid analgesic widely used for relieving severe pain such as that associated with cancer and surgery. However, its repeated administration may lead to the development of antinociceptive tolerance through the activation of spinal glial cells[4]; the administration of glial modulators, such as propentofylline, minocycline, and P2X4 receptor antisense oligonucleotide, may help attenuate this.[5,6]

Yokukansan (Yi-Gan San; YKS), first reported in the *Baoying jin-jing-lu* (written in 1550), is a traditional herbal (Kampo) medicine consisting of seven herbs (Table 1).[7] YKS has been administered to patients who show symptoms such as emotional irritability, neurosis, and insomnia and to infants who suffer from night crying and convulsions.[8] However, YKS has also recently been reported to be effective against pain disorders, such as headache, post-herpetic neuralgia, fibromyalgia, and trigeminal neuralgia.[9,10] Previous studies have demonstrated the antinociceptive effects of YKS in mice models with visceral pain[11] and rat models with chronic constriction injury.[12,13] Moreover, Nakagawa et al[14] reported that the preadministration of YKS daily for 3 weeks attenuates morphine tolerance; however, they did not discuss the mechanism involved sufficiently. We have previously reported that YKS has analgesic effects on chronic inflammatory pain using rat models with adjuvant arthritis, and one of the mechanisms involved was the inhibitory effect of YKS on the activation of microglial cells.[15]

Therefore, in the present study we investigated the inhibitory effect of YKS on the activation of spinal microglia and astrocytes using a rat model with morphine tolerance.

Table 1 – Component galenicals of Yokukansan (YKS; TJ-54)

Component galenicals of Yokukansan (YKS; TJ-54)	
Uncariae cum Uncis ramulus	3.0 g
Cnidii rhizoma	3.0 g
Bupleuri radix	2.0 g
Atratylodis Lanceae rhizoma	4.0 g
Poria	4.0 g
Angelicae radix	3.0 g
Glycyrrhizae radix	1.5 g

The weights show the mixing ratio.

2. Methods

2.1. Animals

This study used male Wistar rats (7 wk old, weighing 190–220 g) that were purchased from Nippon Bio-Supp. Center (Tokyo, Japan). During the study period, the animals were housed in standard plastic cages in our animal facilities at 25 ± 2 °C, 55 ± 5% humidity, and a 12-hour light/dark cycle. Food and water were provided *ad libitum*. All experiments were performed according to the guidelines of the Committee of Animal Care and Welfare of Showa University (certificate number: 02028).

2.2. Administration of YKS

YKS (Lot No. 2110054010; manufactured by Tsumura, Tokyo, Japan; Table 1) was mixed with powdered rodent chow (CE-2; CLEA Japan, Tokyo, Japan) at a concentration of 3% and fed to the YKS-treated rats. The rats that were not treated with YKS were fed powdered chow only. The concentration was chosen based on effective doses of YKS recommended by previous reports.[15]

2.3. Antinociceptive effect of YKS

The rats were randomly divided into a control group (Con; $n = 7$) and a YKS-treated group (YKS; $n = 7$), and thermal hyperalgesia was assessed daily for 10 days by measuring the withdrawal latency using a hot plate test.[16] The rats were placed on a hot plate (Hot plate analgesia meter model 39D; IITC Life Science, Woodland Hills, CA, USA) with the temperature adjusted to 47.5 °C. The latency up to the first sign of paw licking or jumping in response to the heat was measured, and 20 seconds was considered as the cutoff point to avoid tissue damage. The administration of YKS was started immediately after conducting the hot plate test on Day 1.

2.4. Influence of YKS on the antinociceptive effect of morphine

This test was performed to investigate whether YKS had any influence on the antinociceptive effect of a single morphine administration. The rats were randomly divided into a