Available online at SciVarse ScienceDirect
Chinese Herbal Medicines (CHM)
ISSN 1674-6384
Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Effects of Zhengtian Pills on Migraine Headache in Rats via Transient Receptor Potential Vanilloid 1

Jun-hua Sun[1†], Ke-zhu Wang[1†], Hui Fu[1], Zhi Dai[2], Fei-fei Pu[1], Song Yin[3], Tian-xiu Qian[1], Xin-min Liu[1], Xiao-ying Wang[1*]

1. Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, China

2. China Resources Sanjiu Medical & Pharmaceutical Co., Ltd., Shenzhen 518110, China

3. Department of Pharmacy, Maternal and Child Hospital of Xiangtan, Xiangtan 411104, China

ARTICLE INFO

Article history

Received: March 15, 2016

Revised: April 20, 2016

Accepted: May 10, 2016

Available online:

July 6, 2016

DOI:

10.1016/S1674-6384(16)60049-7

ABSTRACT

Objective To investigate the effects and molecular mechanism of Zhengtian Pills (ZTP) on migraine headache. **Methods** All rats were randomly divided into control, positive control, migraine model, low- and high-dose ZTP groups, and glyceryl trinitrate was injected to induce migraine headache. The time of ears turning red, frequency of scratching head, climbing the cage, and head-twitching were used to evaluate rat behaviors. After 10 d administration of ZTP, the expression levels of transient receptor potential vanilloid 1 (TRPV1) both in cortex and hippocampus were determined by Western blotting. **Results** After 2 min of glyceryl trinitrate injection, rats showed headache phenomena that parallels the clinical symptoms of migraine, which peaked in 30 min, and lasted for 60 min. Frequency of head-twitching and numbers of scratching head in glyceryl trinitrate (GTN) group were significantly increased. In contrast, after ZTP (1.08 g/kg, ig) treatment, the numbers of scratching head with fore-limb, hind-limb and the frequency of head-twitching were significantly decreased. Flunarizine (FLU) and low-dose ZTP (0.54 g/kg) also showed a trend to decrease the numbers of scratching head and head-twitching frequency, but no significant difference. Besides, ZTP significantly decreased the up-regulated TRPV1 protein expression level both in cortex and hippocampus. **Conclusion** The present study shows that ZTP could significantly improve the migraine symptoms of headache in rats and TRPV1 might be one of the important molecular mechanisms. This is the first report about the effect of ZTP on TRPV1 protein expression level both in cortex and hippocampus of rats.

Key words

behavior; glyceryl trinitrate; migraine; TRPV1; Zhengtian Pills

[*] **Corresponding author: Wang XY** Tel: +86-10-5783 3494 Fax: +86-10-5783 3228 E-mail: xywang@implad.ac.cn

[†] These authors contributed equally to this work.

Funds: Ministry of Science and Technology (grant No. 2013ZX09201022) and the National Natural Science Foundation of China (grant No. 81373418)

1. Introduction

Migraine is a very common primary headache disorder associated with intermittent attacks and great suffering. Migraine has a prevalence of 10% in the general population and its societal costs are high. Typically, the headache affects one half of the head and is pulsating in nature. The precise mechanisms underlying the pathophysiology of migraine are still elusive (Seng and Seng, 2016; Cameron et al, 2015; Khan et al, 2015). Considerable debate has surrounded the cause and underlying pathology of migraine and several theories have been proposed, for example, depolarization theory, vascular theory, serotonin theory, and hypothyroidism.

Generally, the acute treatment of migraine attacks has been limited to the use of analgesics, combinations of analgesics with caffeine, ergotamines, and the triptans. Some new approaches for the treatment of acute migraine target the calcitonin gene-related peptide, serotonin receptors, and glutamate, $GABA_A$ receptors, or a combination of $5\text{-}HT_{1B/1D}$ receptors and neuronal nitric oxide synthesis also show promise as both acute and preventive therapies. Besides, humanized antibodies against CGRP or CGRP receptor, calcitonin gene-related peptide, non-invasive and invasive neuromodulation approaches were investigated, further studies are needed to define the appropriate candidates for these therapies and none of them has been successful in clinical trials thus far. Collectively, for migraine therapy, the available chemical drugs are either ineffective or poorly tolerated thus far (Diener et al, 2015; Ferrari et al, 2015).

Traditional Chinese medicine (TCM) is one of the oldest healing systems, and herbal medicine is the important part of TCM. The holistic philosophy of TCM shares much with the key ideas of emerging network pharmacology and network biology, and meets the requirements of overcoming complex diseases in a systematic manner. Zhengtian Pills (ZTP), a traditional potential and effective therapeutic tool for migraine and other types of headaches, such as tension headache and sinus headache in China, which consists of 15 medicinal herbs such as *Ramulus Uncariae cum Uncis* (Gouteng), *Paeoniae Alba Radix* (Baishao), *Angelicae Sinensis Radix* (Danggui), *Chuanxiong Rhizoma* (Chuanxiong), *Rehmanniae Radix* (Dihuang), *Saposhnikoviae Radix* (Fangfeng), *Angelicae Dahuricae Radix* (Baizhi), *Notopterygii Rhizoma* et *Radix* (Qianghuo), *Persicae Semen* (Taoren), *Carthami Flos* (Honghua), *Asari Radix* et *Rhizoma* (Xixin), *Angelicae Pubescentis Radix* (Duhuo), *Ephedrae Herba* (Mahuang), *Aconiti Lateralis Radix Praeparata* (Fupian), and *Caulis Spatholobi* (Jixueteng). “Jun-Chen-Zuo-Shi”, also known as sovereign-minister-assistant-courier, was followed to prescribe this component herb according to that of the TCM. ZTP has been used in clinic for over 30 years and holds a great promise for treating headaches in an integrative and holistic way. However, the detailed molecular mechanism was not clear yet. Therefore, in the present study, the effects and molecular mechanism of ZTP on migraine headache will be investigated.

2. Materials and methods

2.1 Animals

Fifty Sprague-Dawley (SD) rats [male, (200 ± 20) g] were provided by Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The animals were housed in standard laboratory conditions [under 12 h light/dark cycles and at a temperature of (23 ± 1) ^oC] and could freely access to food and water. Experimental protocols and procedures were handled in accordance with Chinese Animal Use and Care Committees and executed according to National Animal Law.

2.2 Drugs and reagents

Glyceryl trinitrate injections (No. 20140310) were purchased from Beijing Yimin Pharmaceutical Co., Ltd. and sc given at dose of 10 mg/kg. ZTP was supplied by Sanjiu Medical & Pharmaceutical Company, and was re-suspended in ddH_2O. Flunarizine (FLU) (No. 140414122) was obtained from Xi’an Janssen Pharmaceutical Ltd. and also dissolved in ddH_2O. FLU was ig given at dose of 0.9 mg/kg. All other chemical reagents in our study were of analytical grade.

2.3 Experimental groups

All rats were randomly divided into five groups such as control group (saline), glyceryl trinitrate-induced migraine model (GTN) group, low- and high-dose (0.54 and 1.08 g/kg) ZTP groups, and FLU group (positive control), formed by 10 animals in each group. The animals were prepared as previously described (Wang et al, 2011). After 3 d habituation, rats orally received either ddH_2O (control and GTN groups) or various concentration of ZTP and FLU (0.9 mg/kg) for 10 d. After 30 min of last administration, glyceryl trinitrate (10 mg/kg) was sc given to establish migraine model.

2.4 Glyceryl trinitrate-induced headache in rats

The animals were acclimatized for 3 d in the test chamber before testing. After 10 d of administration, the computer-aided controlling system for open field test was conducted to appraise the general behavior and symptom during 90 min. The general behavior and symptom, including the time of turning red of rats' ears, frequency of scratching head, climbing cage, and head-twitching, which were used to evaluate the different categories of headache in rats. After detection of the basal level of general behavior, glyceryl trinitrate (10 mg/kg) was sc given to duplicate the animal model of migraine, and the GTN rats were immediately placed into open field chambers to detect the numbers of scratching head, climbing cage, and head-twitching. The behaviors and symptoms were real-time recorded and continuously observed at various time intervals.

2.5 Western blotting

After the migraine model was established, the cortex