Brain, Behavior, and Immunity 64 (2017) 180–194

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Full-length Article

The novel and potent anti-depressive action of triptolide and its influences on hippocampal neuroinflammation in a rat model of depression comorbidity of chronic pain

Xiaofan Hu [a,b], Yulin Dong [a], Xiaohang Jin [a], Chunkui Zhang [a], Ting Zhang [a], Jie Zhao [a], Juan Shi [a,*], Jinlian Li [a,*]

[a] Department of Human Anatomy, Histology and Embryology & K. K. Leung Brain Research Centre, Preclinical School of Medicine, The Fourth Military Medical University, Xi'an 710032, China
[b] Department of Orthopedics, Xijing Hospital, The Fourth Military Medical University, Xi'an 710032, China

ARTICLE INFO

Article history:
Received 11 December 2016
Received in revised form 2 March 2017
Accepted 10 March 2017
Available online 12 March 2017

Keywords:
Depression
Chronic pain
Comorbidity
Triptolide
Fluoxetine
Hippocampus
Neuroinflammation

ABSTRACT

Chronic pain and depression frequently coexist in clinical setting, and current clinical treatments for this comorbidity have shown limited efficacy. Triptolide (T10), an active component of *Tripterygium wilfordii* Hook F., has been demonstrated to exert strong analgesic activities in experimental pain models, but whether it possesses anti-depressive actions remains unknown. Using a depression comorbidity of chronic pain rat model induced by spinal nerve ligation (SNL), we investigated the potency of T10 for the treatment of comorbid depression in comparison with a widely used antidepressant, fluoxetine (FLX). Concomitant neuroinflammation changes were also examined in the hippocampus. The results showed that prophylactic and reversal treatments with T10 dose-dependently (30, 100, 300 μg/kg) inhibited the depression-like behaviors (DLB) assessed by the forced swim test, sucrose preference test and body weight measurement. The anti-depressive efficacy of T10 at 300 μg/kg was significantly stronger than that of FLX at 18 mg/kg. T10 at all three doses exhibited more efficient analgesic effects than FLX at 18 mg/kg. The combined application of T10 with FLX markedly augmented the effects of T10 or FLX per se, with the facilitating effects of T10 at 30 μg/kg being most prominent. In addition, nerve injury caused the activation of microglia and p38 MAPK, the upregulation of IL-1β and TNF-α as well as the downregulation of IL-10 in the hippocampus at postoperative week (POW) 3. These neuroinflammatory responses were reversed by subchronic treatment with T10. Taken together, these results demonstrate that T10 possesses potent anti-depressive function, which is correlated with its immunoregulation in the hippocampus. The combination of a low dose of T10 with FLX may become a more effective medication strategy for the treatment of comorbid depression and chronic pain.

1. Introduction

Mental disorders, such as depression, frequently coexist with chronic pain in the clinical setting (Holmes et al., 2013; Miller and Cano, 2009). Epidemiological studies have reported that an average of 52% of pain patients fulfill the criteria for depression and that the mean prevalence of pain in depressed patients reaches 65% (Bair et al., 2003; Korff and Simon, 1996). Comorbid depression contributes to more intense pain, greater pain-related disability, and a poorer response to chronic pain treatment (Holmes et al., 2013; Rayner et al., 2016). Thus, it is necessary to treat not only chronic pain itself but also the depression accompanying the disease. Currently, however, patients who suffer from both chronic pain and depression are particularly difficult to treat (Tunks et al., 2008), and therapeutic strategies for this comorbidity remain fairly limited (Doan et al., 2015). Thus, the search for novel pharmacological agents or medication strategies with a higher potency toward both pain and depression is necessary to improve the treatment of comorbid depression and chronic pain.

Neuroimmune responses are involved in the pathophysiology of both depression (Hodes et al., 2015) and chronic pain (Grace et al., 2014), and neuroinflammation has been regarded as a common mechanism underlying pain-depression comorbidity (Walker et al., 2014). In the past decades, accumulating evidence has demonstrated that peripheral inflammation is associated with both depression and pain, possibly through passive leakage, active transport and neural afferent transduction of local or systemic

* Corresponding authors at: Department of Anatomy, Histology and Embryology & K. K. Leung Brain Research Centre, Preclinical School of Medicine, The Fourth Military Medical University, Xi'an 710032, China.
E-mail addresses: shixjuan@fmmu.edu.cn (J. Shi), jinlian@fmmu.edu.cn (J. Li).

http://dx.doi.org/10.1016/j.bbi.2017.03.005
0889-1591/

cytokines to the brain (Walker et al., 2014). Nevertheless, central inflammation is becoming an increasing focus due to the concomitant immune activation of the central nervous system (CNS) under depression or pain conditions. Recently, increasing evidence has emerged that supraspinal changes in cytokine or chemokine expression can be induced following the establishment of neuropathic pain. The enhancement of inflammatory mediators has been implicated in the affective comorbidities of chronic pain (Fiore and Austin, 2016). Microglia are tissue-resident macrophages in the brain and spinal cord. In neuropathic pain, the over-activated microglia and subsequently activated astrocytes release proinflammatory cytokines, establishing a neuroinflammatory framework and modulating pain processing at both spinal and supraspinal levels (Grace et al., 2014; Tiwari et al., 2014). While the neuroimmune mechanisms in chronic pain have been extensively studied in the spinal cord (Grace et al., 2014), assessments of specific supraspinal areas are rather sparse.

The hippocampus undergoes significant neuroinflammation following chronic pain induced by nerve injury (Fasick et al., 2015). Regardless of the cellular origin, there is growing evidence that pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6, are markedly increased in the hippocampus of animals with chronic pain (Yalcin et al., 2014). Several lines of evidence suggest that microglia in the hippocampus are also chronically activated following the establishment of neuropathic pain and depression induced by spinal cord injury or peripheral nerve injury (Gui et al., 2016; Wu et al., 2014). Based on both preclinical and clinical studies, it has been argued that the emergence of affective disturbances in neuropathic pain are contingent on neuroinflammation in the interconnected hippocampal-medial prefrontal circuitry (Fiore and Austin, 2016). Thus, vigorously targeting supraspinal neuroinflammation, such as those in the hippocampus is likely to be a new avenue to treat debilitating depression comorbidity of chronic pain.

Triptolide (T10), one of the major active components of *Tripterygium wilfordii* Hook F. (TWHF), is a diterpenoid triepoxide ($C_{20}H_{24}O_6$; for chemical structure of T10, please see Fig. S1). It has been shown that this drug is potent for the treatment of various diseases, such as rheumatoid arthritis, nephritic syndrome, lupus, Parkinson's disease (PD) and Alzheimer's disease (AD) via its anti-inflammatory and immunoregulatory actions (Hoyle et al., 2010; Nie et al., 2012; Zheng et al., 2013). Our previous studies have demonstrated that T10 effectively relieves neuropathic pain by inhibiting the activation of microglia and astrocytes in the spinal dorsal horn (Tang et al., 2012; Wang et al., 2012). However, it remains unknown whether T10 is effective for the treatment of depression comorbidity of chronic pain given the common neuroinflammatory pathophysiology of the two symptoms.

In the present study, the effect of T10 on comorbid depression of neuropathic pain was investigated in a rat model of spinal nerve ligation (Kontinen et al., 1999; Li, 2015). For better appraisal, we also compared the anti-depressive and anti-nociceptive effects of T10 with those of fluoxetine (FLX), a commonly used antidepressant, and tested the effects of the combined application of these two drugs. The possible cellular and molecular mechanisms underlying the anti-depressive effects of T10 were also investigated by observation of the neuroinflammation changes in the hippocampus.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (180–220 g) were housed in a temperature-controlled room with free access to food and water at 22–25 °C on a 12-h light/dark cycle. All animal procedures were approved by the Animal Use and Care Committee for Research and Education of the Fourth Military Medical University (Xi'an, China) and carried out in accordance with the National Institutes of Health Guidelines for the Use of Laboratory Animals strictly. All efforts were made to minimize suffering and the number of animals used.

2.2. SNL surgery and evaluation of depression-like behaviors

Spinal nerve ligation (SNL) was performed according to Kim and Chung (Kim and Chung, 1992). Briefly, the rats were anesthetized with 7% chloral hydrate (0.4 ml/100 g, i.p.). A midline incision was made at the L3-S2 level, and the dorsal vertebral plate from L4 to S2 was exposed. After the L6 transverse process was carefully removed, the left L5 spinal nerve was isolated and tightly ligated distal to the dorsal root ganglion (DRG) with 6–0 silk thread. Sham-operated animals were subjected to a similar surgical procedure in which the isolated nerves were not ligated.

The forced swim test (FST), sucrose preference test (SPT) and body weight measurements were employed to investigate the time course of the depression-like behaviors (DLB) induced by SNL (Fig. 1). Because the FST per se can have a considerable influence on animal emotion (Slattery and Cryan, 2012), 4 sets of rats (every set contains naïve, sham and SNL groups with each group of 6–7 rats) were submitted to body weight, SPT and FST measurements sequentially at the end of postoperative week (POW) 1, 2, 3 and 5, respectively. One set of animals were tested only once at respective time point.

2.3. CUMS model of depression

The procedure used for the chronic unpredicted mild stress (CUMS) model of depression was performed as described previously with minor modifications (Luo et al., 2013; Willner et al., 1987). Rats in the CUMS group were subjected to daily varied stressors for seven consecutive days (Table S1). Subsequently, weekly rotation of the same stimuli was continued for another four weeks. The stressors applied included the following: cage tilting (45 degrees) for 12 h, cold water swimming (5 min at 4 °C), wet bedding for 24 h, tail restraint for 5 min, water and food deprivation for 12 h, change to a single cage for 12 h, placement in a cage with strange rats for 12 h, exposure to noise for 8 h, and inversion of the light/dark cycle for 24 h. Control rats were housed under the condition with the same breed but with no exposure to the above stressors or contact with the stressed animals.

2.4. Drug administration

T10 obtained from the Fujian Academy of Medical Sciences (Fujian, China) was dissolved in dimethylsulfoxide (DMSO, Sigma, St. Louis, MO) and then diluted in saline to a concentration of 1 μg/μl, with the final concentration of DMSO as 0.4%. FLX (Sigma-Aldrich) was dissolved in phosphate-buffered saline to obtain a stock solution of 5 mg/ml (PBS, pH 7.4). The doses of T10 administered intraperitoneally and FLX by gavage were adopted from the literature (David et al., 2009; Wang et al., 2012).

Three patterns of drug application were employed in the present study: acute, prophylactic and reversal (Fig. 2). In the acute paradigm (Fig. 2A), the rats in the SNL group and sham group were intraperitoneally (i.p.) injected with vehicle or T10 at doses of 30, 100 or 300 μg/kg, at the end of POW 3 (21 d). One hour after injection, the rats were sequentially subjected to the SPT and the FST pretest with a 30-min interval. On the second day (22 d), vehicle or varied doses of T10 were again delivered, and the FST was conducted after one hour. For the subchronic prophylactic treatment (Fig. 2B), drugs were given after the nerve injury but before the