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Complete Freund's adjuvant–induced acute inflammatory pain could be attenuated by triptolide via inhibiting spinal glia activation in rats

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ARTICLE INFO

Article history:
Received 26 June 2013
Received in revised form
12 November 2013
Accepted 15 November 2013
Available online 23 November 2013

Keywords:
Triptolide
Inflammatory pain
Glia
pERK

ABSTRACT

Background: Inflammatory pain is one of the most common clinical symptoms, mechanical allodynia and thermal hypersensitivities are associated with proinflammatory cytokines, and proinflammatory cytokine antagonists could alleviate the hypersensitivity. Previous studies showed that a traditional Chinese medicine ingredient, triptolide could inhibit inflammatory cytokines; however, it was still unknown whether triptolide had beneficial effects on treating inflammatory pain.

Materials and methods: The effects of triptolide on Complete Freund's Adjuvant–induced acute inflammatory pain were investigated using behavioral tests. The activation of spinal glia was morphologically observed by immunofluorescent histochemistry. The levels of OX42, glia fibrillary acidic protein, and phosphorylated extracellular signal–regulated kinase in the spinal cord were detected by Western blot, and the messenger RNA levels of interleukin 1β, interleukin 6, and tumor necrosis factor alpha were detected by real-time polymerase chain reaction.

Results: These results demonstrate that the triptolide effectively attenuates inflammatory pain induced by Complete Freund's Adjuvant, the underlying mechanism may regulate the phosphorylated extracellular signal–regulated kinase signaling pathway and inhibit the spinal glia activation, and then downregulate the proinflammatory cytokines; the triptolide may be clinically useful as a drug of anti-inflammatory pain.

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http://dx.doi.org/10.1016/j.jss.2013.11.1087

Conclusions: In the present study, we first reported that repeated systemic administration of triptolide could safely prevent and reverse inflammatory pain. The triptolide may serve as a new potential compound for developing safe therapeutics for patients suffering inflammatory pain.

1. Introduction

Pain followed tissue lesions is one of the most common clinical symptoms, including bone fracture, peripheral neuropathy, and so forth. Acute inflammatory pain is much suffering and turned to be intractable also poor of effective treatment [1]. Inflammatory pain produces mechanical allodynia and thermal hypersensitivity, which could be related to the inflammatory mediators released from inflammatory or adjacent tissues; patients presented to be decreased pain threshold and increased response to stimulus, resulting in nociception [2,3]. But the peripheral factors were not enough for expliciting the hypersensitivity of acute inflammatory pain; the release of a series of chemical signals would alter the threshold of nociceptors [4] and the excitability of spinal neurons [5]. Mechanical allodynia and thermal hypersensitivities are associated with proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β [6,7], and proinflammatory cytokine antagonists could alleviate the hypersensitivity [8,9]. The suppression of proinflammatory cytokines can partially reduce inflammation; although these drugs are currently available for clinical use, they are not highly effective and have significant side effects [10,11]. Many plants and their compounds used in traditional medicine might be useful in alleviating inflammation and inflammatory pain [12,13]. As a traditional Chinese medicine, The *Tripterygium wilfordii* Hook. f. often used for treating inflammatory diseases [14], which is a vine-like member of the celastraceae plant family, and triptolide was the major active ingredient. Previous studies have shown that the triptolide could inhibit TNF-α, IL-1β, and nitric oxide production in microglia [14]. The triptolide could effectively protect neurons from inflammatory damage through inhibiting microglial activation [15]. These data indicated that triptolide could significantly repress the immune response; however, it was still unknown whether triptolide had beneficial effects in treating inflammatory pain especially the acute ones. In the present study, we tested the triptolide-inhibited hypersensitivity and further tried to explore the underlying mechanism.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (180–220 g) were used in the study; rats were housed in a temperature-controlled environment at 22°C–25°C and 12-h light/dark cycle. The animals were free to food and water. The Animal Care and Use Program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. The minimum number of animals were used to demonstrate consistent effects.

The study was approved by the Ethical Committee of Animal Research at the PLA 302 Hospital, Beijing, China. All experiments were conducted in accordance with the Institutional Committee for the Care and Use of Animals and guidelines from the Ethical Standards for Investigations of Experimental Pain in Conscious Animals [16] and the International Association for the Study of Pain.

2.2. Drug injection

Thirty-six rats were randomly divided into six groups: saline, saline + triptolide (T), saline + U0126, Complete Freund's adjuvant (CFA), CFA + T, and CFA + U0126 ($n = 6$ for each group). The rats in the CFA and CFA + T groups were subcutaneously injected with 150 μL of CFA (Sigma, St. Louis, MO) in the plantar surface of the right hind paw while under light ether anesthesia. The CFA injection immediately induces local inflammation, paw swelling, and pain, which persist for at least 2 wk after injection [17]. The 18 rats in the saline, saline + T, and saline + U0126 groups were injected with 150 μL of physiological saline under identical conditions. The rats in the saline + T and CFA + T groups were treated with triptolide (100 μg/kg) during the period of day 2 (2 d before CFA injection) to day 7 (7 d after injection) [18]. The rats in the saline + U0126 and CFA + U0126 groups were parallelly treated by intraperitoneal injection of U0126 (300 mg/kg; Sigma). The rats in the CFA and saline control groups were intraperitoneally injected with same amount of saline.

2.3. Behavioral tests

2.3.1. Paw withdraw latency in response to noxious thermal stimuli

Paw withdraw latency (PWL) in response to noxious thermal stimuli was assessed using an RTY-3 radiant heat stimulator (Xi'an Fenglan Instrumental Factory, Xi'an, China) by those blinded to the group assignments [3]. This device produces radiant heat by directing a beam of light to the plantar surface of the hind paw; the light is extinguished on paw withdrawal. The rats were placed in plastic boxes on a glass plate for at least 30 min before testing. The time from initiation of the light beam to paw withdrawal was noted as PWL. Three trials on the same paw were performed with intervals of at least 5 min. To prevent tissue damage, radiant heat was administered for a maximum of 20 s. The rats were habituated to the testing environment for 3 d before baseline testing. The rats were tested on two successive days before CFA injection to determine a baseline value for each animal. The PWLs were then tested at 2, 6, 12, and 24 h and 2–7 d after CFA injection.