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Orientin and neuropathic pain in rats with spinal nerve ligation

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ABSTRACT

Neuropathic pain affects patients worldwide. The therapeutic effects of current methods are still poor. This study was performed to investigate the neuro-protective effect of orientin in rats with spinal nerve ligation (SNL). In this study, the paw mechanical withdrawal threshold (PWT) and the paw thermal withdrawal latency (PWL) behavioral assays indicated that orientin alleviated the warm and mechanical allodynia in rats with SNL. The enzyme-linked immunosorbent assay (ELISA) showed that orientin suppressed the levels of pro-inflammatory cytokines interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor alpha (TNF-α) and increased the levels of anti-inflammatory cytokine interleukin-10 (IL-10). Malondialdehyde (MDA) levels were down-regulated while superoxide dismutase (SOD) and glutathione (GSH) levels were up-regulated by orientin. OX42 and GFAP immune fluorescent staining results demonstrated that orientin inhibited the activation of microglia and astrocytes in rats with SNL. Western blot analysis indicated that the neuroprotective effect of orientin was mediated by inhibition of Toll-like receptor 4 (TLR4)/nuclear factor kappa B (NF-kappa B) signaling pathway. This study suggested that orientin is a promising neuroprotective agent suitable for therapy for neuropathic pain.

1. Introduction

Neuropathic pain is a type of pain initiated by a primary lesion or dysfunction of the nervous system. It affects 30% of the population worldwide [1]. The causes of neuropathic pain are numerous and include iatrogenic injury, traumatic injury, cancer compression, chemotherapy drugs, diabetes, and even viral disease [2]. Neuropathic pain is a thorny problem in clinical settings, and it is often only minimally sensitive to the usual therapies [3]. Neuropathic pain leads to a wide variety of problems in human body function, such as limitation of activity or disability [4]. However, the therapeutic effects of current treatment remain limited, although a large amount of progress has been made in the past few decades in the medical field [5]. Complete elimination of the sensation of pain and complete restoration of function are only rarely the goals of such projects [6]. New approaches with fewer side effects and more pronounced curative effects are sorely needed.

Orientin (Fig. 1A) is isolated from alcoholic leaf extract of *Phyllostachys parvifolia*, a medical plant used in folk medicine worldwide. Orientin has exhibited extensive medical value in the past, and this has drawn attention from a large number of scholars. Many studies have demonstrated that orientin possesses adipogenesis inhibitive [7], antioxidant [8], anti-nociceptive [9], and anti-icrobial activity [10] in cell lines or animal models. In addition, the most significant pharmacological action of orientin is anti-inflammatory ability in vitro and in vivo [11]. Orientin has been found to suppress inflammation in human umbilical vein endothelial cells (HUVECs) [12,13], RAW 264.7 cells [14], vascular inflammatory mice [15,16], and kidney inflammation [17]. Additionally, orientin also protected subjects from myocardial ischemia reperfusion injury via targeting apoptosis and the regulation of autophagy [18,19].

For the pharmacological application on nervous system disease, orientin exerted neuroprotective effect on hydrogen peroxide-induced apoptosis in SH-SY5Y cell lines with few cytotoxic effect in vitro [20]. Orientin promoted cognitive recovery and alleviated oxidative stress in Aβ1-42-induced Alzheimer's disease rat model [21]. Orientin has been found to accelerate alleviation of depression-like behavior in chronically stressed mice [22]. Orientin improved noise-induced cognitive impairments in a mouse model [23]. Here, we hypothesized that

Abbreviations: SNL, spinal nerve ligation; ELISA, enzyme-linked immune sorbent; IF, immune fluorescent; PWL, paw thermal withdrawal latency; PWT, paw mechanical withdrawal threshold; TNF-α, tumor necrosis factor alpha; IL-6, interleukin-6; IL-1β, interleukin-1β; IL-10, interleukin-10; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; GSH, glutathione; TLR4, Toll-like receptor 4; NF-kappa B, nuclear factor kappa B

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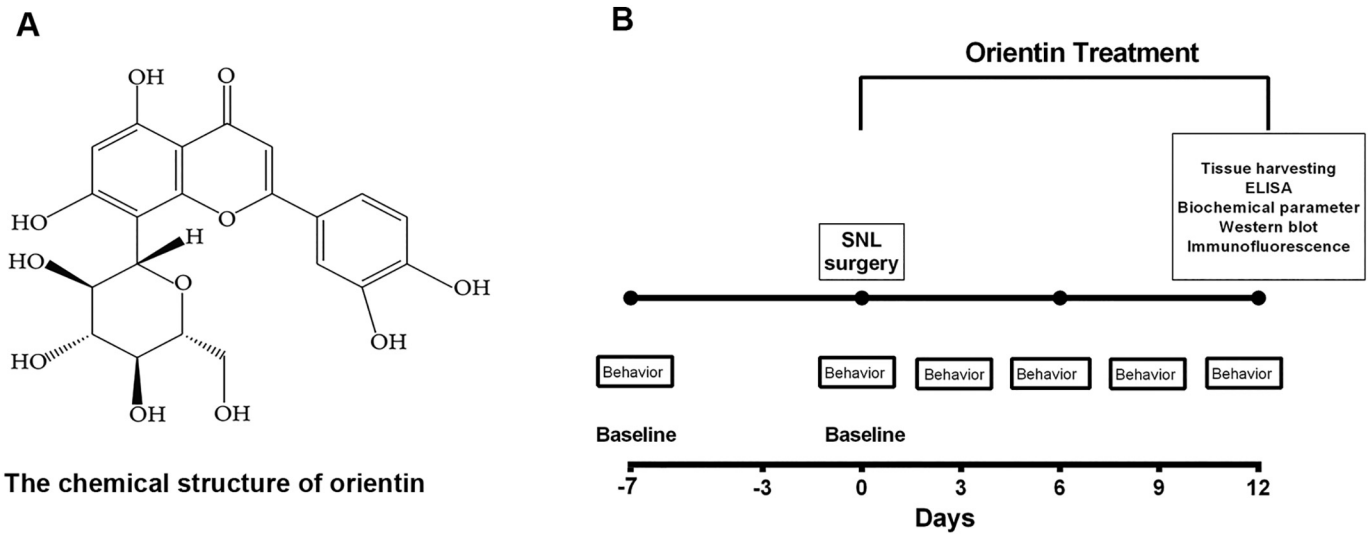

Fig. 1. (A) The chemical structure of orientin. (B) The protocol of animal study.

orientin has a beneficial effect on neuropathic pain. In this way, the present work was performed to determine whether orientin promoted recovery on spinal nerve ligation model at the behavioral level and to investigate underlying mechanical interactions.

2. Material and methods

2.1. Chemicals

The enzyme-linked immunosorbent assay (ELISA) kits and the lipid peroxidation assay kits were purchased from Beyotime Institute of Biotechnology (Beyotime, Haimeng, China). The orientin standard (purity > 98%) were obtained from Shanghai Rochen Life Science Corporation (Shanghai, China). Antibodies were purchased from Cell Signaling Technology (CST, Danvers, MA, USA) except where otherwise mentioned. Other unmentioned reagents were of analytical grade.

2.2. Animals

The animal study was conducted according to the Declaration of Helsinki, the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC, National Research Council of America, 1996) and the Use of Laboratory guidelines issued by the Chinese Council on Animal Care. Animal experiments were approved by the Institutional Animal Care and Use Committee of Fudan University (Grant Number: 2017K1005). A total of 50 Sprague-Dawley (SD) rats (200–220 g, specific pathogen free (SPF), healthy and all males) were obtained and housed at temperatures of 20–25 °C, humidity of 40 ± 5% and 12 h light/12 h dark cycle environment at SPF grade. Five rats in same group were maintained in a cage with dimensions of 485 × 350 × 200 mm with bedding of sawdust and free to feed on pathogen free rat/mouse pelleted diet and water.

2.3. Groups protocol

The rats were assigned randomly into 5 groups (n = 10): (1) sham operation group (SO), (2) model group (MD), (3) low dose orientin group (10 mg/kg, LO), (4) medium dose orientin group (20 mg/kg, MO), (5) high dose orientin group (40 mg/kg, HO), (6) positive drug group (PD). The SO group underwent sham surgery operation while the other groups underwent SNL. The baseline data were recorded 7 days before and after the SNL operation using behavioral tests. After the SNL operation, orientin was administered to the LO, MO, and HO groups. The orientin was dissolved in 5% DMSO/saline intraperitoneally injected into the left flank of the rats in the 3 orientin groups once per day at the doses given above. Orientin was administered for 12 days after the SNL operation. The equivalent volume of sterilized 5% DMSO/saline was injected into the MD group. The pregabalin was dissolved in sterilized saline and injected intravenously (i.v.) at 10 mg/kg daily in the PD group [24,25]. The protocol of animal study was indicated in Fig. 1B.

2.4. SNL model operation

The SNL surgery operation was conducted as procedures reported with minor amend [26]. Concisely, after anesthesia and trim of lower lumbar and sacral section at right side, a dorsal midline incision was performed from spinal L3–S2 levels to expose nerve around L5–L6. The right L5 spinal nerve was ligated using a 6–0 silk suture. The operation was conducted carefully to avoid L4 and L6. The skin and inner muscular were sutured soon after the nerve ligation. After the operation, each rat was given penicillin via intravenous injection at 0.2 million units once per day in the following 3 days to avoid infection. The SO group underwent similar manipulation but no ligation.

2.5. Behavioral assay

The behavioral assay was performed to measure the paw mechanical withdrawal threshold (PWT) and the paw thermal withdrawal latency (PWL) which represents mechanical and thermal allodynia following previously report [27]. The behavior assay was analyzed using calibrated nylon Von Frey filaments (Stoelting, Wood Dale, IL, US) on an elevated mesh floor. For the PWT test, the ipsilateral hind paw was pressed with ascending stiffness from 0.4–26 g (0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, and 26.0 g) at 12:00 a.m. to 3:00 p.m. after 30 min of accommodation. The measurement was repeated 3 times at 5 min intervals. Withdrawal or the paw within 6–8 s was considered a positive reaction during each measurement. For the PWL test, rats were put on a 6 mm–thick glass plate, and the PWL was recorded 3 times at intervals longer than 5 min using a thermal radiation instrument (BME-410C, Boerni Corporation, Tianjin, China). The PWT and PWL were recorded as the mean of data collected 3 times. The behavioral test was performed the same day as the SNL surgery to record the baseline data of rats in each group. The behavioral tests of PWT and PWL were conducted every 3 days from the initial orientin administration to the end.