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Research paper

Silencing of FKBP51 alleviates the mechanical pain threshold, inhibits DRG inflammatory factors and pain mediators through the NF-kappaB signaling pathway

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ABSTRACT

Neuropathic pain is chronic pain caused by lesions or diseases of the somatosensory system, currently available analgesics provide only temporal relief. The precise role of FK506 binding protein 51 (FKBP51) in neuropathic pain induced by chronic constriction injury (CCI) is not clear. The purpose of the present study was to investigate the effects and possible mechanisms of FKBP51 in neuropathic pain in the rat model of CCI. Our results showed that FKBP51 was obviously upregulated in a time-dependent manner in the dorsal root ganglion (DRG) of CCI rats. Additionally, silencing of FKBP51 remarkably attenuated mechanical allodynia and thermal hyperalgesia as reflected by paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) in CCI rats. Moreover, knockdown of FKBP51 reduced the production of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6), nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) expression in the DRG of CCI rats. Furthermore, we revealed that inhibition of FKBP51 greatly suppressed the activation of the NF-kappaB (NF-κB) signaling in the DRG of CCI rats. Interestingly, similar to the FKBP51 siRNA (si-FKBP51), ammonium pyrrolidinedithiocarbamate (PDTC, an inhibitor of NF-κB) also alleviated neuropathic pain and neuro-inflammation, indicating that knockdown of FKBP51 alleviated neuropathic pain development of CCI rats by inhibiting the activation of NF-κB signaling pathway. Taken together, our findings indicate that FKBP51 may serve as a novel therapeutic target for neuropathic pain.

1. Introduction

Neuropathic pain is characterized by allodynia, hyperalgesia and spontaneous pain resulting from damage or dysfunction of the nervous system (Sorge et al., 2012). It is caused by diverse factors, including inflammation, metabolic disorders, and surgical traumas (Ochoa, 2009). In recent years, neuropathic pain has become a common problem in medical care and affects a broad population worldwide. The main obstacle hampering the development of effective therapeutics is the inability to target underlying mechanisms of neuropathic pain. In order to develop novel therapeutic strategies for neuropathic pain, it is essential to gain a better understanding of the molecular mechanisms underlying its cause.

Neuropathic pain is a common chronic pain, and includes spontaneous pain, allodynia, hyperalgesia and hypersensitivity, and pain disorders (Gilron et al., 2015). The increasing amount of evidence has implied that neuro-inflammation is associated with the development of neuropathic pain (Ellis and Bennett, 2013). Mounting evidences have revealed that pro-inflammatory cytokines including TNF-α and IL-1β, play important roles in neuropathic pain (Cha et al., 2012; Yano et al., 2013; Lu et al., 2014). The release of inflammatory cytokines, IL-1β, TNF-α, and IL-6 contribute to the development of neuropathic pain (Moalem and Tracey, 2006).

FK506-binding proteins are members of the immunophilin family of proteins. Those immunophilins associated to the 90-kDa-heat-shock protein, Hsp90, have been proposed as potential modulators of signaling cascade factors chaperoned by Hsp90 (Lagadari et al., 2016). FK506 binding protein 51 (FKBP51) is a high molecular weight FK506-binding protein involved in the regulation of diverse biological processes (Hausch, 2015). Polymorphisms in the FKBP51 gene, a regulator of steroid hormone signaling, influence the severity of musculoskeletal pain symptoms after trauma. It is reported that FKBP51 is crucial for the full development and maintenance of long-term pain states. FKBP51 regulates chronic pain by modulation of glucocorticoid signaling. It is a central mediator of chronic pain, likely in humans as well as rodents, and is a new pharmacologically tractable target for the treatment of

Abbreviations: FKBP51, FK506 binding protein 51; CCI, chronic constriction injury; PWT, paw withdrawal threshold; PWL, paw withdrawal latency; DRG, dorsal root ganglion

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long-term pain states (Scammell et al., 2001; Maiarù et al., 2016a, b). However, under neuropathic pain conditions, the role of FKBP51 is still unclear. Thus, in the present study, we established a rat model of neuropathic pain induced by chronic constriction injury (CCI), and investigated the novel role of FKBP51 in regulating the development of neuropathic pain in rats after CCI. Our research was conducted to gain greater insight into the function of FKBP51 in the peripheral nervous system, provided a theoretical basis for regulation of FKBP51 in neuropathic pain.

2. Materials and methods

2.1. Chronic constriction injury (CCI) model

Experiments were conducted using adult male Sprague-Dawley rats weighing 200–250 g that were purchased from the Experimental Animal Center of Hebei Medical University. All surgical and experimental procedures in this study were admitted by the Animal Use and Care Committee for Research and Education of Hebei Medical University. Animal treatments were performed in accordance with the Guidelines of the International Association for the Study of Pain (Zimmermann, 1983). Sprague-Dawley rats underwent surgery for the CCI model of neuropathic pain. For the CCI surgery, rats were anesthetized with sodium pentobarbital (40 mg/kg, 10% solution) (Kukkar et al., 2014). The left common sciatic nerve was exposed at mid-thigh level, and 4 ligatures were tied loosely around it with about 1 mm between ligatures. The incision was closed in layers, and the animal recovered. Rats in the sham group underwent sham surgery in which the left sciatic nerve was exposed, but not ligated.

2.2. SiRNA injection

FKBP51 siRNA (si-FKBP51) and negative control siRNA (si-NC) were purchased from QIAGEN (Germantown, Philadelphia, USA). Rats were divided into 5 groups with 12 rats each group: Sham group, CCI group, si-NC + CCI group, si-FKBP51 + CCI group and ammonium pyrrolidinedithiocarbamate (PDTC) + CCI group. For intrathecal injection, the rats of si-NC + CCI group, si-FKBP51 + CCI group were injected intrathecally with si-NC or si-FKBP51 (6 μg in 10 μL annealing buffer) on day 4 after CCI surgery for 3 consecutive days. PDTC (Sigma, St. Louis, MO) was freshly dissolved daily in 0.9% sterile, isotonic saline, and the rats was injected intrathecally with PDTC (1000 pmol/d) on day 4 after CCI surgery for 3 consecutive days.

2.3. Evaluation of thermal hyperalgesia and mechanical allodynia

To assess neuropathic pain, mechanical and thermal allodynia were measured. Animals were habituated to the testing environment daily for at least three days before baseline testing. Behavioral analysis (n = 5) was performed three days before surgery and from one day to four weeks after the CCI surgery. The room humidity and temperature were kept stable for all experiments. Rats were placed in elevated perspex cages (15 × 10 × 10 cm) with a wire mesh floor and allowed to acclimatize for 10–30 min prior to testing. To assess mechanical allodynia, the paw withdrawal threshold (PWT) to mechanical stimulation of the left hind paw was assessed using von Frey hairs (North Coast Medical, San Jose, USA). A series of von Frey hairs (0.2–8.5 g) were pressed perpendicularly (for 2 s) onto the plantar surface of the hind paw (four times for each hair), and the mechanical PWT was calculated using a threshold tracking algorithm (Chaplan et al., 1994). Thermal hyperalgesia was assessed by the paw withdrawal latency (PWL) to radiant heat according to a previously reported method (Hargreaves et al., 1988). After acclimation, the heat source was positioned under the glass floor directly beneath the hind paw. The intensity of the thermal stimulus was adjusted to achieve an average baseline PWL of approximately 10 s. A digital timer automatically recorded the duration between the start of stimuli and PWL. A 20 s cutoff was used to avoid tissue damage. Each paw was measured in turn with an interval of > 5 min between each measurement.

Table 1
Primer sequences of target genes are shown in Table 1.

Gene	Sequence (5′–3′)
TNF-α	F:TCAGTTCCATGGCCCAGAC
	R:GTTGTCTTTGAGATCCATGCCT
IL-1β	F:CCCAACTGGTACATCAGCACCTCTC
	R:CTATGTCCCGACCATTGCTG
IL-6	F:GATTGTATGAACAGCGATGATGC
	R:AGAAACGGAACTCCAGAAGACC
NGF	F:AGGGCAGACCCGCAACATC
	R:GGTGGAGGCTGGGTGCTAAAC
BDNF	F:CATAAGGACGCGGACTTGTACA
	R:AGCAGAGGAGGCTCCAAAGG
β-actin	F:TCAGGTCATCACTATCGGCAAT
	R:AAAGAAAGGGTGTAAAACGCA

2.4. Real-time PCR (RT-PCR) analysis

Total RNA was isolated from the dorsal root ganglion (DRG) using TRIzol Reagent (Invitrogen Inc., Carlsbad, CA, USA) according to the manufacturer's protocol. The concentration of total RNA was determined using an Ultraviolet Spectrophotometer (Eppendorf, Hamburg, German). cDNA was synthesized using a High-Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, USA) following the manufacturer's protocol. RT-PCR was performed using SYBR Green I (Applied Biosystems, USA), and carried out on an ABI Prism 7700 analyzer (Applied Biosystems, Warrington, UK). The relative quantification was normalized to the internal reference gene. The expression of target gene was calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences of target gene were shown in Table 1.

2.5. Western blot analysis

Proteins were extracted from the DRG of CCI rats and protein concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo, USA). Rats were sacrificed at different time points after operation after administration of an overdose of chloral hydrate, and the DRG tissues post operation were excised and instantly frozen at − 80 °C until they were assayed. Frozen DRG tissue samples were weighed and then minced with eye scissors on ice. Proteins in cytoplasmic and nuclear fractions were extracted by Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, China) according to the manufacturer's instruction. The Bradford assay (Bio-Rad) was used to determine the concentration of protein. Samples were then subjected to SDS-PAGE and then transferred to PVDF membrane (Millipore, Billerica, MA). The membrane was then blocked with 5% non-fat dried skim milk or bovine serum albumin (polyclonal antibodies) in TBST (20 mM Tris, 150 mM NaCl, and 0.05% Tween-20). After 2 h at room temperature, the membranes were incubated with appropriate primary antibodies at 4 °C overnight. After incubating with second antibody for 1 h at room temperature, the signals were developed with enhanced chemiluminescence (ECL; Advansta, Manlo Park, California, USA) and visualized on X-ray films. Quantitative analysis was performed using Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA). The relative protein expression levels were normalized to GAPDH or β-actin.

2.6. ELISA assay

After the last injection, the DRG was harvested and homogenized in lysis buffer. The concentrations of TNF-α, IL-6 and IL-1β in lumbar DRG were measured using corresponding ELISA kits (R & D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.