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Inhibition of interleukin-6 function attenuates the central sensitization and pain behavior induced by osteoarthritis

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ABSTRACT

Chronic pain is the most prominent and disabling symptom in the patients with osteoarthritis (OA), and the underlying mechanism largely remains unclear. Interleukin-6 (IL-6), a proinflammatory cytokine, is critically involved in the development and maintenance of central sensitization in several rodent models of chronic pain. The present study aims to elucidate the IL-6 mediated neurological adaptation in dorsal horn in the rat with monosodium iodoacetate (MIA) – induced OA. Significant upregulation of IL-6 expression was detected in the dorsal horn in the modeled rats. Blockade of IL-6 function by tocilizumab markedly suppressed the activation of astrocytes and microglia in the ipsilateral dorsal horn, reduced c-Fos immunoreactivity in dorsal horn neurons, and attenuated the upregulation of glutamate receptor subunits GluR1 and NR2B in dorsal horn in the rats with MIA-induced OA. It was further reported that administration of tocilizumab significantly improved the performance in weight-bearing test and mitigated the mechanical allodynia in the modeled rats. These data illustrated spinal IL-6 mediated mechanism underlying the chronic pain, and proposed the potential therapeutic effect of tocilizumab on the chronic pain in the setting of OA.

1. Introduction

Osteoarthritis is characterized by a progressive loss of articular cartilage, subchondral bone lesions and synovitis, and currently serves as one of the most common cause of disability in elderly people (Arden and Nevitt, 2006). Despite of complicated variance of clinical phenomena, chronic pain is the most prominent and disabling symptom in the patients with OA (Hunter et al., 2008). While the well-characterized structure changes in the joint initiate the development of chronic pain, increasing evidences demonstrated that the spinal and supraspinal neurological adaptation (central sensitization) essentially contributes to the spreading and facilitation of the pain sensation in the OA patients (Gwilym et al., 2009; Arendt-Nielsen et al., 2010). Currently it remains challenging to well understand the mechanisms that drive this chronic pain state, which largely hampers the optimistic management of the chronic pain in the OA patients.

IL-6 is a proinflammatory cytokine secreted by immune cells including T cells, macrophages (microglia in the central nerve system) (Scheller et al., 2014; Tanaka and Kishimoto, 2014) and astrocyte (Gruol, 2016), and exerts wide-ranging biological effects by interacting with the non-signaling membrane-bound IL-6 receptor on target cells in the physiological and pathological circumstances (Scheller et al., 2014; Tanaka and Kishimoto, 2014). Activation of IL-6 receptor may, *via* association with the signal transducing membrane protein gp130, stimulate several intracellular signaling including the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT), mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathways (Boulanger et al., 2003; Heinrich et al., 2003). Accumulating evidences have demonstrated the critical role of IL-6 in the development and maintenance of behavioral hypersensitivity in numerous rodent models of pain (Zhou et al., 2016). The expression of IL-6 was elevated in the peripheral dorsal root ganglia and several brain regions involved in the nociceptive sensory processing, and administration of IL-6 could cause mechanical allodynia or thermal hyperalgesia in the rodents (Guptarak et al., 2013; Ding et al., 2016). Inhibition of IL-6 receptor function by neutralizing antibody exhibited potential effect to attenuate the thermal hyperalgesia and mechanical allodynia in several settings of chronic pain (Guptarak et al., 2013; Zhou et al., 2016). IL-6 was reported to essentially contribute to nociceptor sensitization and

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central sensitization, and induce behavioral sensitization in the neurological disorders with chronic pain (Brenn et al., 2007; Vazquez et al., 2012; Ding et al., 2016). Currently, the involvement of IL-6 in dorsal horn in the development and maintenance of chronic pain in the setting of OA remains unclear. Converging data suggested the potential participation of IL-6 in cognitive function and neuroinflammatory diseases by altering synaptic transmission and neuron excitability (Gruol, 2015; Vezzani and Viviani, 2015). Enhanced glial fibrillary acidic protein (GFAP) levels, dendritic excitatory synaptic potentials and somatic population spikes were found in the hippocampus of those IL-6 transgenic mice (Nelson et al., 2012). Glutamate is the primary excitatory neurotransmitter, and its N-methyl-D-aspartic acid (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors are critical molecules responsible for the neuroplasticity formation in chronic pain (Katano et al., 2011). The present study therefore aims to study the involvement of IL-6, as well as its possible influences on the NR2B subunits of NMDA receptors, and GluR1 subunits of AMPA receptors in the setting of chronic pain in the rat model of OA.

2. Materials and methods

2.1. Animal model and treatment

All experimental animal protocols were approved by the Institutional Animal Care and Use Committee at Shichuan University, and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Male Sprague Dawley rats weighing 250–300 g were purchased from Chengdu Dashuo Biological Technology Co., Ltd., one of the certified suppliers of experimental animals for Shichuan University. All animals were housed in temperature- and light-controlled (12 h light/dark cycle) conditions with free access to food and water.

Experimental OA model was induced by injection of MIA into the knee of rats, as previously reported (Yu et al., 2013), which was the most commonly used OA model for the study of joint pain, and well mimics the pathological changes and pain of osteoarthritis in humans (Combe et al., 2004; Pomonis et al., 2005). Briefly, the rats were anesthetized with 10% chloral hydrate in saline, and 3 mg MIA (Sigma-Aldrich, USA) in a total volume of 25 μl saline was injected intra-articularly through the patellar ligament of the left knee with a 30-gauge needle and a Hamilton microsyringe. Meanwhile, same volume of saline was applied in the knee in a different cohort of rats.

Tocilizumab, a recombinant humanized IL-6 monoclonal antibody with a concentration 20 μg/μl, was purchased from Chugai Pharma Manufacturing Co., Ltd. The tocilizumab was further dilated with saline to the concentration 1 μg/μl. After intra-articularly injection of the MIA, a single 10 μl tocilizumab (10 μg) was directly intrathecally injected *via* a pencil-point microsyringe as previously described (Mestre et al., 1994; De la Calle and Paino, 2002). The accurate injection into the subarachnoid space was verified with the successfully withdrawing cerebrospinal fluid and the injection induced tail-flick.

2.2. ELISA measurement of IL-6

The content of IL-6 in dorsal horn (L4-L6) was measured with the commercial ELISA kits (EMD Millipore, Billerica, MA) following the manufacturer's instruction. Briefly, the rats were rapidly killed and the L4-L6 spinal dorsal horn was rapidly collected and homogenized in lysis buffer containing protease inhibitors, and the insoluble pellet was separated out by centrifugation. IL-6 concentration in the supernatants was measured with a spectrophotometer (ABCDS, Germany). All samples were processed in triplicate. Data were obtained from 3 independent experiments.

2.3. Immunoblotting

Immunoblotting was performed in the dorsal horn tissues as previously reported. In brief, rats were deeply anesthetized with sodium pentobarbital (Nembutal, 100 mg/kg, i.p.), and the L4-L6 spinal dorsal horn was rapidly collected. The dorsal horn tissues were punched and homogenized in lysis buffer with protease inhibitors. Total proteins were obtained by centrifugation at 16,000 g for 15 min at 4 °C. 10 μg of protein was subjected to SDS-PAGE and transferred onto a nitrocellulose membrane. Blots were probed with anti-GluR1 antibody (1:1000; Cell signaling, USA), anti-NR2B antibody (1:1000; Cell signaling), anti-IL-6 antibody (1:1000; Cell signaling), or anti-β-actin antibody (1:1000; EMD Millipore). The immunosignals were detected with the enhanced chemiluminescence assay, and the Image J software was used to quantify the protein band intensities. The amounts of GluR1, NR1 and IL-6 proteins were normalized to that of β-actin, which was compared among the different groups.

2.4. Immunohistochemical analysis

Immunohistochemical staining in spinal dorsal horn section was performed as previously reported (Xu et al., 2016). Briefly, the rats in all groups were deeply anesthetized with sodium pentobarbital (75 mg/kg, i.p.) and perfused through the ascending aorta with saline followed by cold 4% paraformaldehyde in 0.1 m PB. The spinal cord segments (L4-L5) were collected, fixed in 4% paraformaldehyde overnight, and then cryoprotected in 30% sucrose solution. Transverse spinal cord sections (25 μm) were obtained in a cryostat. Primary antibodies against microglial marker CD11b (1:500, ABcam, USA), astrocyte marker GFAP (1:500, ABcam, USA), and neuronal marker NeuN (1:500, Abcam, USA) were co-incubated with IL-6 antibody (1:500; Abcam, USA) or c-Fos antibody (1:500; Abcam, USA) overnight at 4 °C, and FITC- and Cy3- conjugated secondary antibody was then applied. Five optical sections from each rat were randomly selected and quantified using a Zeiss, imager Z2 microscope by an investigator who was blinded to the origin of tissue. The staining intensities were examined in a standardized area of spinal dorsal horn. After the background fluorescence was subtracted, fluorescence image stacks were quantified for both fluorescence intensity and relative area occupied by labeled objects. Negative control sections were incubated with secondary antibody alone to account for the autofluorescence from the dorsal horn itself and nonspecific fluorescence from secondary antibody.

2.5. Pain behavioral testing

Weight-bearing asymmetry of the OA-induced ipsilateral limb with an incapacitance meter (IITC Life Sciences, CA, USA) was measured in all groups as described previously (Sagar et al.,; Pomonis et al., 2005; Yu et al., 2013). In brief, the rats were placed in an angulated Perspex container, and each hind paw rested on a separate transducer pad. The force exerted by each hind limb was measured and averaged over a 5 s period. Changes in weight-bearing asymmetry were evaluated one day before and 2 weeks after MIA or saline injection. The percentage of the weight placed on the left hind limb was determined and compared among the different groups (Sagar et al.,; Pomonis et al., 2005; Yu et al., 2013).

Mechanical withdrawal threshold was tested by an experimenter blinded to treatment groups during the daylight hours (10:00 A.M. to 4:00 P.M.). The 50% paw withdrawal threshold in response to a series of 8 von Frey hairs (0.41–15.10 g) was examined by the up-down method, as described previously (Xu et al., 2016), beginning with a filament with a bending force of 2.0 g. During the study, the rats were placed under clear acrylic cages atop a wire mesh floor. The filaments were applied to the paw just below the pads with no acceleration at a force just sufficient to produce a bend and held for 6 s. A quick flick or