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ASPIRIN-TRIGGERED LIPOXIN A_4 ATTENUATES MECHANICAL ALLODYNIA IN ASSOCIATION WITH INHIBITING SPINAL JAK2/STAT3 SIGNALING IN NEUROPATHIC PAIN IN RATS

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Abstract—Aspirin-triggered Lipoxin A_4 (ATL), as a Lipoxin A_4 (LXA_4) epimer, is endogenously produced by aspirin-acetylated cycloxygenase-2 (COX-2) and plays a vital role in endogenous anti-inflammation via the LXA_4 receptor (ALX). Recent investigations have indicated that spinal neuroinflammation and the activation of the Janus Kinase 2 (JAK2)/Signal Transducers and Transcription Activators 3 (STAT3) signaling pathway are involved in neuropathic pain states. However, the effect of ATL on neuroinflammation and JAK2/STAT3 signaling in chronic constriction injury (CCI)-induced neuropathic pain in rats has not been well-studied. The present study demonstrated the anti-inflammatory and analgesic effect of ATL on neuropathic pain and assessed the role of spinal JAK2/STAT3 signaling on the effect of ATL. Intrathecal administration of ATL significantly attenuated mechanical allodynia via spinal ALX and inhibited the upregulation of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) on day 7 of CCI surgery. In addition, ATL markedly suppressed the upregulation of p-STAT3 induced by the neuropathic pain. Blockade of JAK2–STAT3 signaling with intrathecal administration of the JAK2 inhibitor AG490 or the STAT3 inhibitor S3I-201 clearly reduced mechanical allodynia and the upregulation of pro-inflammatory cytokines in CCI rats. Interestingly, inhibition of JAK2/STAT3 signaling via ATL or the specific signaling inhibitor (AG49, S3I-201) further promoted the increased expression of suppressor of cytokine signaling 3 (SOCS3) mRNA in the spinal cord induced by CCI surgery. Taken together, our results suggested that the analgesic effect of ATL was mediated by inhibiting spinal JAK2/STAT3 signaling and hence the spinal neuroinflammation in CCI rats.

Key words: aspirin-triggered Lipoxin A_4, spinal cord, neuropathic pain, inflammation, JAK2/STAT3.

INTRODUCTION

Neuropathic pain induced by peripheral nerve injury is a prevalent, persistent, and debilitating problem. Recent studies have indicated that spinal neuroinflammation may play a critical role in the pathological states of neuropathic pain (Myers et al., 2006; Kiguchi et al., 2012). In addition, neuroinflammation may be induced by the activation of spinal glial cells or neurons in pain states (Echeverry et al., 2008; Benarroch, 2010; Gwak et al., 2012). Mounting evidence has indicated that spinal glial activation contributes to the secretion of pro-inflammatory cytokines that are crucial for nociceptive transmission and the occurrence of painful behavior (Guo et al., 2007; Cao and Zhang, 2008; Kawasaki et al., 2008; Ji et al., 2009). Inhibition of neuroinflammation mediated by glial cells has been shown to attenuate inflammatory or neuropathic pain (Deleo et al., 1996, 1997; Jia et al., 2009; Lu et al., 2013).

Lipoxins (LXs) were the first identified family of endogenous ''braking signals'' in inflammation in vivo (Serhan and Chiang, 2002; Serhan, 2005; Jin et al., 2012). LXs and aspirin-triggered Lipoxin A_4 (ATL) are eicosanoids, which are derived from the sequential lipoxygenase (LO) metabolism of arachidonic acid. Lipoxin A_4 (LXA_4) binds to its specific G-protein-coupled receptor, known as the LXA_4 receptor (ALX), and butoxy carbonyl-Phe-Leu-Phe-Leu-Phe (Boc-2) is an effective antagonist of ALX (La et al., 2001; Perretti et al., 2002;

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Abbreviations: ALX, LXA_4 receptor; ATL, aspirin-triggered Lipoxin A_4; CCI, chronic constriction injury; COX-2, cycloxygenase-2; CSF, cerebral spinal fluid; CT, threshold cycle; DMSO, dimethyl sulfoxide; FITC, fluorescein isothiocyanate; JAK2, Janus Kinase 2; LO, lipoxygenase; LXA_4, Lipoxin A_4; LXs, lipoxins; NS, NaCl solution; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SCI, spinal cord injury; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SNL, spinal nerve ligation; SOCS, suppressor of cytokine signaling; STAT3, Signal Transducers and Transcription Activators 3.

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Gavins et al., 2003; Haworth et al., 2008). ATL, as a LXA_4 epimer, is endogenously produced by aspirin-acetylated cycloxygenase-2 (COX-2) (Claria and Serhan, 1995). ATL is more stable than LXA_4 since it is more resistant to further conversion, and shares many anti-inflammatory activities and exhibits the same receptor affinity of native LXA_4. Recent studies have shown a vital role of LXA_4 and ATL in pain processing and neuroinflammation (Serhan et al., 1995; Svensson et al., 2007). In our previous study, the analgesic effect of ATL was demonstrated in the bone cancer pain and chronic constriction injury (CCI)-induced neuropathic pain in rats (Hu et al., 2012; Li et al., 2013). Compared with LXA_4, ATL displayed more effective activity in inflammatory pain and bone cancer pain. However, there have been a few reports regarding the effect of ATL on mechanical allodynia of CCI-induced neuropathic pain.

The Janus Kinase 2 (JAK2)–Signal Transducers and Transcription Activators 3 (STAT3) pathway is one important signaling pathway downstream of cytokine receptors, which plays a variety of roles in the development of inflammatory disease (Seavey and Dobrzanski, 2012; O'Shea and Plenge, 2012). The activation of astrocytes involved in the induction of neuropathic pain is dependent on the phosphorylation states of JAK2–STAT3 (Herrmann et al., 2008; Svensson and Brodin, 2010; Gao and Ji, 2010; Tsuda et al., 2011). Inhibition of the JAK2–STAT3 pathway by the JAK2 inhibitor AG490 relieves the pain states induced by spinal nerve ligation (SNL) injury and decreases central inflammation (Gorina et al., 2005; Satriotomo et al., 2006; Dominguez et al., 2008). S3I-201, a novel selective inhibitor of STAT3, preferentially inhibits STAT3 DNA-binding activity and diminishes STAT3 phosphorylation (Siddiquee et al., 2007; Pang et al., 2010). However, whether the JAK2–STAT3 pathway is involved in the analgesic effect of ATL on neuropathic pain is still unclear.

Suppressor of cytokine signaling (SOCS) families have been shown to act as negative feedback inhibitors of the JAK2/STAT3 signaling pathway, which are involved in the signaling regulation of cytokines (Dominguez et al., 2010; Tamiya et al., 2011; Tang et al., 2012). SOCS1 and SOCS3 relieve neuroinflammation in IFN-β-treated astrocytes and regulate inflammation of sciatic nerve injury (Qin et al., 2008; Girolami et al., 2010). SOCS2-deficient mice have uncontrolled production of proinflammatory cytokines, and SOCS2 is a crucial intracellular mediator of anti-inflammatory ATL activity in vivo (Machado et al., 2006). However, it is not clearly known whether SOCS in the spinal cord is associated with the analgesic effect of ATL on neuropathic pain.

On the basis of these findings, our study investigated the role of the JAK2–STAT3–SOCS pathway in the analgesic effect of ATL in CCI rats. We observed the effect of the spinal injection of ATL on CCI-induced neuropathic pain, and assessed the role of JAK2–STAT3 pathway.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats weighing 200–220 g were used in all experiments. All animals were kept under controlled conditions (a temperature-controlled room (24 ± 0.5 °C), a 12:12-h light cycle (07:00–19:00 light), with free access to food and water). All animal experiments were performed in accordance with the IASP's guidelines for pain research (Zimmermann, 1983).

Surgical procedures

Neuropathic pain was induced according to the protocol previously described by Bennett and Xie (1988). Briefly, rats were anesthetized with 0.04 g/kg i.p. chloral hydrate. Under aseptic conditions, the right common sciatic nerve was exposed at the level of the middle thigh via a blunt dissection. Proximal to the trifurcation, the nerve was carefully freed from the surrounding connective tissue and four chromic cat gut ligatures (4-0, Shanghai Pudong Jinhuan Medical Products Co, LTD, China) were tied loosely proximal to the sciatica's trifurcation at 1-mm intervals. After hemostasis was confirmed, the incision was closed in layers and disinfected with penicillin. The animals were allowed to recover from surgery and then housed one per cage with free access to water and standard laboratory chow. Sham surgeries were performed by exposing the right sciatic nerve without ligation.

Drug administration

ATL (a LXA_4 analog), LXA_4 (5(S),6(R)-LXA_4) and BOC-2 (a ALX antagonist) were used in the experiment. ATL and LXA_4 were purchased from Merck (Darmstadt, Germany) and BOC-2 was purchased from ICN Pharmaceuticals (Basingstoke, United Kingdom). ATL and LXA_4 were dissolved in 10% dimethyl sulfoxide (DMSO) or 0.9% NaCl solution (NS), and BOC-2 was dissolved in 10% DMSO. Different doses of ATL (50, 100, 200 ng) and BOC-2 (50 μg/kg) were used in the experiment and controls were treated with the same amount of vehicle. Two doses of the JAK2 inhibitor AG490 (1 μg and 5 μg dissolved in 8% DMSO) were intrathecally administered at 2 h pre-surgery and on days 1 and 2 post-CCI surgery. Intrathecal doses of the STAT3 inhibitor S3I-201 (10 μg and 100 μg dissolved in 8% DMSO) were administered on post-operation day 7 of CCI surgery. To perform the intrathecal injection, the rats were anesthetized with 2% isoflurane. After shaving the lumbar region and sterilizing it with 70% ethanol, the animals were given a lumbar puncture at the L4–L5 interspace using a 0.5-in. 30-gauge needle. Drugs were administered into the cerebral spinal fluid (CSF) space via lumbar puncture, as previously reported (Xu et al., 2006). A 29-gauge microinjection syringe needle was inserted via the L5–L6 interspace. The correct subarachnoid positioning of the tip of the needle was verified by a

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