Journal of Neuroimmunology 319 (2018) 71–79

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Anti-IL-6 receptor antibody improves pain symptoms in mice with experimental autoimmune encephalomyelitis

Kenichi Serizawa*, Haruna Tomizawa-Shinohara, Mayu Magi, Kenji Yogo, Yoshihiro Matsumoto

Product Research Department, Chugai Pharmaceutical Co., Ltd, Shizuoka, Japan

ARTICLE INFO

Keywords:
IL-6
Anti-IL-6 receptor antibody
Experimental autoimmune encephalomyelitis
Neuropathic pain
Microglia

ABSTRACT

Background and aims: Chronic pain is a prevalent symptom in patients with autoimmune encephalomyelitis such as multiple sclerosis and neuromyelitis optica. Although IL-6 is involved in various inflammatory and immune diseases, the roles of IL-6 in autoimmune-related pain have not been clarified. Therefore, we examined the effect of anti-IL-6 receptor antibody (MR16-1) on the pain sensitivity of experimental autoimmune encephalomyelitis (EAE) mice.

Materials and methods: EAE was induced in female C57BL/6J mice by subcutaneous immunization with myelin oligodendrocyte glycoprotein 35–55 emulsified in adjuvant (Day 0). Pertussis toxin was intravenously administered at Days 0 and 2. Mice were sequentially scored for clinical symptoms of EAE. [Exp. 1] MR16-1 was intraperitoneally administered on Days 0 or 3. Sensitivity to pain was measured by the von Frey test (Days 7, 14, 20). The spinal cord was isolated and assessed by immunohistochemistry. [Exp. 2] MR16-1 was intraperitoneally administered on Day 12 when significant pain had already occurred. Pain assessment was conducted before the immunization, on Day 12 and after EAE onset. And then, spinal cord was isolated and flow cytometry was performed.

Results: [Exp. 1] MR16-1 prevented the increase in clinical score and sensitivity to pain in EAE mice. Immunohistochemical analysis showed that $Iba1^{+}$ microglia were increased in the spinal cord of EAE mice, and were reduced by MR16-1. [Exp. 2] Administration of MR16-1 on Day 12 also reduced sensitivity to pain under EAE onset. Flow cytometry showed that $CD45^{low}CD11b^{+}$ microglia were increased in the spinal cord of EAE mice, and that this increase was inhibited by MR16-1.

Conclusion: These findings suggest that MR16-1 can decrease mechanical allodynia in EAE mice through inhibition of microglial activation and proliferation in the spinal cord.

1. Introduction

Chronic pain is a major symptom associated with demyelinating autoimmune diseases of the central nervous system (CNS), such as multiple sclerosis and neuromyelitis optica. It has been reported that approximately 50–80% of patients with multiple sclerosis or neuromyelitis optica feel pain (Kanamori et al., 2011; Osterberg et al., 2005; Zhao et al., 2014), and neuropathic pain is the most prevalent and difficult to treat in these patients (Kessler et al., 2016; Svendsen et al., 2005).

Neuropathic pain arises from central sensitization (Woolf, 1983), which is triggered partly by the activation of glial cells (Ikeda et al., 2012; Tsuda et al., 2005) and inflammation (Kawasaki et al., 2008) in the spinal cord. Activating microglia releases brain-derived neurotrophic factor (BDNF), which in turn disinhibits the endogenous inhibitory system or enhances excitatory synaptic transmission in the dorsal horn neurons of the spinal cord (Coull et al., 2005; Ulmann et al., 2008). These pathological changes convert innocuous inputs to a nociceptive signal output, leading to pain hypersensitivity. It is also suggested that proinflammatory cytokines such as interleukin-6 (IL-6) can play a critical role in neuropathic pain (Zhou et al., 2016). IL-6 increased in the spinal cord of peripheral nerve injury model (Arruda et al., 1998; Winkelstein et al., 2001). Mechanical allodynia was attenuated in the spinal nerve lesion model (Ramer et al., 1998) and chronic constriction injury model (Murphy et al., 1999) using IL-6 knockout mice. Intrathecal injection of IL-6 induced allodynia in normal (non-operated) rats (DeLeo et al., 1996), and peripheral nerve injury-induced mechanical allodynia was attenuated by the spinal

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; GFAP, glial fibrillary acidic protein; MOG, myelin oligodendrocyte glycoprotein

* Corresponding author at: Product Research Department, Chugai Pharmaceutical Co., Ltd, 1-135 Komakado, Gotemba, Shizuoka 412-8513, Japan.
E-mail address: serizawakni@chugai-pharm.co.jp (K. Serizawa).

https://doi.org/10.1016/j.jneuroim.2018.03.017
Received 27 December 2017; Received in revised form 12 March 2018; Accepted 26 March 2018

injection of anti-IL-6 antibody (Arruda et al., 2000). Anti-IL-6 receptor antibody also relieved neuropathic pain in spinal cord injury mice (Murakami et al., 2013). IL-6 mainly activates the JAK/STAT transduction pathway, and STAT3 plays an essential role in IL-6 signaling in the CNS (Imada and Leonard, 2000). Dominguez et al. demonstrated that intrathecal injection of anti-IL-6 antibody prevented accumulation of phospho-STAT3 in the spinal cord microglia and that inhibiting the STAT3 pathway attenuated mechanical allodynia in the spinal nerve injury model (Dominguez et al., 2008). In addition to the central mechanism of IL-6 *via* STAT3 pathway in the spinal cord, Kawasaki et al. demonstrated that IL-6 reduced the frequency of spontaneous inhibitory postsynaptic currents and GABA-induced currents in the spinal cord (Kawasaki et al., 2008).

Experimental autoimmune encephalomyelitis (EAE) is a well-established animal model of the CNS autoimmune disease, and causes widespread CNS inflammation, demyelination, and locomotor impairments (Baxter, 2007). Recently, some studies have demonstrated that EAE mice immunized with myelin oligodendrocyte glycoprotein 35–55 ($MOG_{35–55}$) peptide show neuropathic pain behavior, glial activation and inflammation such as lymphocyte migration and an increase in proinflammatory cytokines including IL-6 (Dutra et al., 2013; Olechowski et al., 2013, 2009). Although it has been demonstrated that anti-IL-6 receptor monoclonal antibody inhibits the development of clinical symptoms such as paralysis in EAE mice (Serada et al., 2008), the effects of the anti-IL-6 receptor monoclonal antibody on the neuropathic pain of EAE mice remained unclear. In the present study, therefore, we examined the analgesic effect of anti-IL-6 receptor monoclonal antibody in EAE mice immunized with $MOG_{35–55}$ peptide. The relationship between the clinical symptoms and the analgesic effects were also explored in mice with EAE.

2. Materials and methods

2.1. Animals

Female C57BL/6J mice (7 weeks old, Charles River Laboratories Japan, Inc., Kanagawa, Japan) were used. All mice were fed ordinary laboratory chow and allowed free access to water under a constant light and dark cycle of 12 h. All animal procedures were conducted in accordance with the *Guidelines for the Care and Use of Laboratory Animals* at Chugai Pharmaceutical Co., Ltd., and all experimental protocols were approved by the Animal Care Committee of the institution and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health.

2.2. Experimental design

Experimental autoimmune encephalomyelitis (EAE) was induced by subcutaneous immunization with 200 μL of an emulsion containing $MOG_{35–55}$ (Peptide International, Louisville, KY, USA) in complete Freund's adjuvant (Difco Laboratories, Detroit, MI, USA) supplemented with *Mycobacterium tuberculosis* extract H37Ra (Difco Laboratories) on Day 0. In addition, mice received intravenous pertussis toxin (List Biological Laboratories, Campbell, CA, USA) on Days 0 and 2. Control mice were treated with complete Freund's adjuvant and saline alone.

2.3. Experiment 1

EAE mice treated with 400 μg $MOG_{35–55}$ and 300 ng pertussis toxin were intraperitoneally administered MR16-1 (8 mg/mouse) or vehicle just after immunization (Day 0) or on Day 3, and tissues were harvested on Day 20. Assignment of clinical scores and assessment of pain were performed before immunization and on Days 7, 14, and 20 (Fig. 1A).

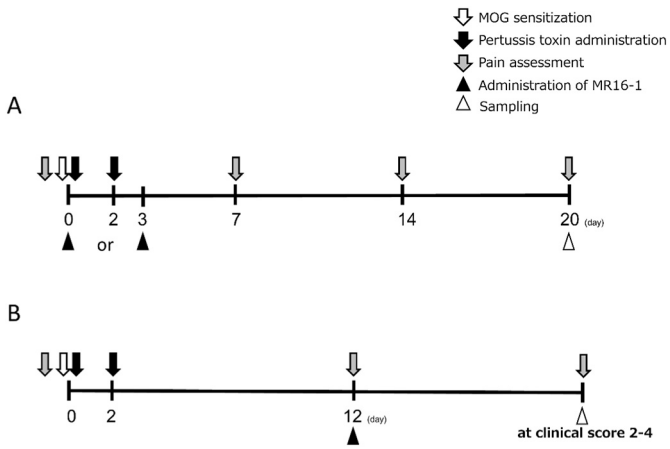

Fig. 1. Outline of the experimental designs.

2.4. Experiment 2

To examine the effect of delayed treatment with MR16-1 for a longer period than in Experiment 1, EAE induction method was modified. EAE mice treated with 50 μg $MOG_{35–55}$ and 250 ng pertussis toxin were intraperitoneally administered MR16-1 (8 mg/mouse) or vehicle on Day 12. From a week later, pain assessment and tissue collection were performed in onset EAE mice (Clinical score, 2–4). Pain assessments were also conducted before immunization and on Day 12 (Fig. 1B).

Graphical representation of study designs for treatment with MR16-1 at Day 0 or 3 (A, Experiment 1) and at Day 12 (B, Experiment 2). (A) MR16-1 or vehicle was intraperitoneally administered just after MOG immunization (Day 0) or on Day 3, and pain assessment was performed before immunization, and on Days 7, 14, and 20. Sampling was performed on Day 20. (B) MR16-1 or vehicle was intraperitoneally administered on Day 12 after MOG immunization. Pain assessment was performed before immunization, on Day 12, and after EAE onset (at clinical score 2–4).

2.5. Clinical score assessment

Clinical symptoms of EAE were scored according to the following scale: 0, no disease; 1, limp tail; 2, hind limb weakness; 3, hind limb paresis; 4, hind limb paralysis; 5, hind limb and fore limb paralysis; 6, moribundity and death.

2.6. Pain assessment

Calibrated von Frey filaments (0.04–2.0 g force) were used to evaluate mechanical allodynia. Mice were placed individually in dark plastic boxes on an elevated wire-mesh bottom plate which allowed full access to the paws. Calibrated von Frey filaments were applied to the plantar surface of the hind paws of mice and the 50% paw withdrawal threshold was determined by using the up–down method (Chaplan et al., 1994).

2.7. Immunohistochemistry

Mice were anaesthetized with isoflurane, and transcardial perfusion was carried out with 20 mL of cold phosphate-buffered saline (PBS) followed by 20 mL of cold 4% paraformaldehyde. The L3–L5 segment of the lumbar spinal cord was removed, post-fixed in 4% paraformaldehyde, and placed in a 30% sucrose solution overnight at 4 °C. Samples were embedded in optimal cutting temperature (OCT) compound, and frozen slices of spinal cord (10 μm thick) were obtained with a cryostat. Spinal cord slices were stained by using the following primary