Pharmacology, Biochemistry and Behavior 126 (2014) 13–27

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Establishment and characterization of an optimized mouse model of multiple sclerosis-induced neuropathic pain using behavioral, pharmacologic, histologic and immunohistochemical methods

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ARTICLE INFO

Article history:
Received 3 June 2014
Received in revised form 25 August 2014
Accepted 6 September 2014
Available online 16 September 2014

Keywords:
Analgesia
Experimental autoimmune encephalomyelitis (EAE)
Mechanical allodynia
Multiple sclerosis (MS)
Neuroinflammation
Neuropathic pain

ABSTRACT

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that causes debilitating central neuropathic pain in many patients. Although mouse models of experimental autoimmune encephalomyelitis (EAE) have provided insight on the pathobiology of MS-induced neuropathic pain, concurrent severe motor impairments confound quantitative assessment of pain behaviors over the disease course. To address this issue, we have established and characterized an optimized EAE-mouse model of MS-induced neuropathic pain. Briefly, C57BL/6 mice were immunized with $MOG_{35–55}$ (200 μg) and adjuvants comprising Quil A (45 μg) and pertussis toxin (2 × 250 ng). The traditionally used Freund's Complete Adjuvant (FCA) was replaced with Quil A, as FCA itself induces CNS neuroinflammation. Herein, EAE-mice exhibited a mild relapsing–remitting clinical disease course with temporal development of mechanical allodynia in the bilateral hindpaws. Mechanical allodynia was fully developed by 28–30 days post-immunization (p.i.) and was maintained until study completion (52–60 days p.i.), in the absence of confounding motor deficits. Single bolus doses of amitriptyline (1–7 mg/kg), gabapentin (10–50 mg/kg) and morphine (0.1–2 mg/kg) evoked dose-dependent analgesia in the bilateral hindpaws of EAE-mice; the corresponding ED_{50}s were 1.5, 20 and 1 mg/kg respectively. At day 39 p.i. in EAE-mice exhibiting mechanical allodynia in the hindpaws, there was marked demyelination and gliosis in the brain and lumbar spinal cord, mirroring these pathobiologic hallmark features of MS in humans. Our optimized EAE-mouse model of MS-associated neuropathic pain will be invaluable for future investigation of the pathobiology of MS-induced neuropathic pain and for efficacy profiling of novel molecules as potential new analgesics for improved relief of this condition.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory-demyelinating disease of the central nervous system disease (CNS) characterized by motor, sensory and cognitive deficits (Compston and Coles, 2008). Pain is a frequently occurring and disabling symptom of MS that has a prevalence in the range of 29–86%, and that may occur even during the early stages of the disease (O'Connor et al., 2008; Solaro and Uccelli, 2011). Pain associated with MS may be broadly classified into nociceptive (e.g. musculoskeletal pain) and neuropathic pain, with MS-induced neuropathic pain regarded as one of the most disabling disease symptoms due to its chronic nature, poor relief by currently available analgesics and complex pathobiology (Khan and Smith, 2014; O'Connor et al., 2008; Truini et al., 2013). MS-induced neuropathic pain is reportedly underpinned by the direct and/or indirect effects of demyelinating lesions in the CNS, and so it is a "central neuropathic pain" (CNP) condition (O'Connor et al., 2008; Solaro et al., 2013). CNP associated with MS is mostly observed in the lower extremities (legs) and is termed dysesthetic extremity pain (Khan and Smith, 2014). It is mostly bilateral, ongoing in nature and characterized by burning, pricking and stabbing sensations (Osterberg and Boivie, 2010; Osterberg et al., 2005; Svendsen et al., 2003). Clinically, this type of CNP has a life-time prevalence in the range of 12–28% (Nurmikko et al., 2010). It may be evident during MS disease onset, and it persists during the disease course with variable intensities (Hadjimichael et al., 2007; Indaco et al., 1994; Osterberg et al., 2005).

The most significant limitation of previously published myelin oligodendrocyte glycoprotein (MOG)-induced EAE rodent models of neuropathic pain is severe motor impairment such that hindlimb paralysis confounds the interpretation of behavioral pain assessments (Khan and Smith, 2014). Hence, most studies that have investigated the pathobiology of neuropathic pain using EAE-mouse models have been restricted

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http://dx.doi.org/10.1016/j.pbb.2014.09.003
0091-3057/

to the early phase of EAE disease prior to the development of confounding motor deficits (reviewed in Khan and Smith, 2014). Additionally, EAE-mouse models induced with $MOG_{35–55}$ using FCA as adjuvant develop chronic-progressive disease without remissions (Constantinescu et al., 2011). In the clinical setting, relapsing–remitting MS (RR-MS) is the predominant disease phenotype affecting ~85% of patients, characterized by symptom worsening (relapses) followed by partial or complete recovery periods (remission) (Compston and Coles, 2008; MSIF, 2014).

Hence, the present investigation was designed to establish an optimized MOG-induced relapsing–remitting EAE mouse model of MS-induced neuropathic pain that is not confounded by severe motor impairment. Specifically, we used saponin from Quillaja (Quil A) bark to replace the commonly used adjuvant, Freund's Complete Adjuvant (FCA) in the EAE immunization protocol. Quil A has been used previously to establish a mild remitting–relapsing EAE mouse model for investigating therapies aimed at attenuating MS disease relapse (Peiris et al., 2007). Importantly, the doses of Quil A and pertussis toxin used herein were each carefully titrated to produce a reproducible, mild relapsing–remitting EAE clinical disease course accompanied by a temporal development of mechanical allodynia in the bilateral hindpaws but without confounding motor deficits. In EAE-mice with fully developed mechanical hypersensitivity in the hindpaws, single bolus doses of amitriptyline, gabapentin and morphine produced dose-dependent analgesia; the ED_{50}s were 1.5, 20 and 1 mg/kg respectively. Additionally, on day 39 p.i. when mechanical allodynia was fully developed in the bilateral hindpaws of EAE-mice (but not sham-mice), there was marked demyelination and glial cell (microglia and astrocytes) activation in the sections of the brain and lumbar spinal cord, mimicking these hallmark features of MS and CNP states in humans.

2. Materials and methods

2.1. Animals

Female C57BL/6 mice aged 4–6 weeks were purchased from The University of Queensland Biological Resources (UQBR). Mice were housed in groups of four to eight per cage in a temperature-controlled facility (22 ± 1 °C) with a 12 h/12 h light/dark cycle. Standard rodent chow and water were available *ad libitum*. Behavioral testing was performed during the light cycle between 0900 and 1600 h. Experimental protocols were approved by the Animal Ethics Committee of The University of Queensland and experiments complied with the requirements of the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2004; NHMRC, 2013).

2.2. EAE immunization and experimental design

The EAE disease induction protocol involved subcutaneous (s.c.) injection of 200 μg of myelin oligodendrocyte glycoprotein peptide 35–55 ($MOG_{35–55}$; MEVGWYRSPFSRVVHLYRNGK; Mimotopes, Clayton, VIC, Australia) that was mixed with Quillaja saponin (Quil A) (Sigma-Aldrich, Sydney, NSW, Australia) solution prepared in 100 μl phosphate-buffered saline (PBS). The mixture containing MOG_{35-55} and Quil A was injected in four equal aliquots (25 μl) administered into both flanks and shoulder regions. Three different doses of Quil A (15, 30 and 45 μg) were assessed. Mice also received either 200 or 250 ng of pertussis toxin (Sigma-Aldrich, Sydney, NSW, Australia) in PBS (1 ng/μl) by intraperitoneal (i.p.) injection on day 0 (first day of immunization) and day 2 (48 h later). Sham-mice received the corresponding doses of Quil A and pertussis toxin only and age-matched control-mice received no treatments. General health and body weights of all animals were assessed prior to immunization and once-daily thereafter until study completion. The EAE immunization protocol and the number of mice used in each cohort of each experiment undertaken to identify the optimal immunization protocol, are summarized in Table 1 and outlined below.

2.2.1. Establishment and optimization of EAE-mouse model of MS-induced neuropathic pain

2.2.1.1. Optimization of Quil A dose (Experiment-1). Three cohorts of female C57BL/6 mice were used to assess the optimal dose of Quil A for establishing remitting–relapsing (RR) EAE-disease and to define the temporal profile for the development of mechanical allodynia in the hindpaws (Table 1). All assessments were performed in a blinded manner.

2.2.1.2. Optimization of pertussis toxin dose (Experiment-2). Three cohorts of female C57BL/6 mice were used to identify the optimal dose of pertussis toxin for robust establishment of RR-EAE in mice and the temporal profile for development of mechanical allodynia in the hindpaws (Table 1). All assessments were performed in a blinded manner.

2.2.2. Pharmacological characterization of optimized EAE-mouse model of MS-induced neuropathic pain (Experiment-3)

The optimized immunization protocol identified in Cohort-3 mice from Experiment-2 (see Section 3.2.2 for further details) was used for the induction of EAE disease in Cohort-1 mice of Experiment-3. Clinical signs were assessed once-weekly and these EAE-mice were used at 30–55 days p.i. for pharmacologic characterization of our optimized model of MS-induced neuropathic pain. EAE-mice with fully developed mechanical allodynia in the bilateral hindpaws were administered single bolus doses of amitriptyline, gabapentin, morphine or vehicle in a blinded manner, and analgesic dose-response curves were generated (see Section 2.5 for further details). In Cohort-2, age-matched control (non-immunized) female C57BL/6 mice were used to assess the extent to which the highest doses of gabapentin, amitriptyline and morphine administered to Cohort-1 EAE-mice, produced motor deficits using an automated gait analysis system (Catwalk™XT) (see Section 2.6 for further details). Gait analysis was performed by a blinded tester.

2.2.3. Histological and immunohistochemical characterization of our optimized EAE-mouse model of MS-induced neuropathic pain (Experiment-4)

The optimized immunization protocol identified and verified in previous experiments (Cohort-3 of Experiment-2 and Cohort-1 of Experiment-3), was used to further assess our optimized RR-EAE mouse model exhibiting mild clinical disease and concurrent temporal development of mechanical allodynia in the bilateral hindpaws. Sham-mice received the same immunization protocol except that MOG was omitted. EAE-mice from Experiment- 4 were euthanized on day 39 p.i., a time when mechanical allodynia was fully developed in the bilateral hindpaws (PWTs ≤ 1.0 g). The corresponding groups of sham- and age-matched control mice (non-immunized) were also euthanized and their CNS tissues were collected for blinded *ex vivo* histologic and immunohistochemical comparison with the corresponding CNS sections from our optimized RR-EAE mouse model of MS-induced neuropathic pain (see Section 2.7 for further details).

2.3. Clinical disease scoring

EAE and sham-mice in each cohort were assessed once-daily over a 50–60 day experimental period in a randomized blinded manner using the following clinical disease scoring paradigm. 0, normal behavior; 0.5, limpness of the distal tail region and hunched appearance; 1, completely limp tail or developing weakness in the hindlimbs; 1.5, limp tail and distinct hindlimbs weakness recognized by unsteady gait and poor grip of hindlimbs during hanging on cage underside; 2, limp tail with unilateral partial hindlimb paralysis; 2.5, Limp tail and partial paralysis of bilateral hindlimbs; 3, complete paralysis of bilateral hindlimbs; 3.5, complete bilateral hindlimbs paralysis and unilateral forelimb paralysis; and 4, Quadriplegia. EAE disease was regarded as present if clinical scores were ≥1 whereas clinical scores ≤0.5 were indicative of no disease or disease remission (Peiris et al., 2007).