Journal of Neuroimmunology 274 (2014) 38–45

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Laquinimod exerts strong clinical and immunomodulatory effects in Lewis rat experimental autoimmune neuritis

Kalliopi Pitarokoili [a,*,1], Björn Ambrosius [a,1], Lisa Schrewe [a], Liat Hayardeny [b], Michael Hayden [b], Ralf Gold [a,**]

[a] Department of Neurology, St. Josef Hospital, Ruhr-University of Bochum, Germany
[b] Teva Pharmaceutical Industries Ltd, 5 Bazel Street, Petah Tiqva 49131, Israel

ARTICLE INFO

Article history:
Received 19 May 2014
Received in revised form 3 June 2014
Accepted 17 June 2014

Keywords:
Autoimmune neuropathies
Laquinimod
Experimental autoimmune neuritis
Autoimmunity
Inflammation
Immunotherapy

ABSTRACT

Laquinimod is an immunomodulatory drug with neuroprotective potential. We used the animal model of experimental autoimmune neuritis (EAN) in the Lewis rat to study the effects of laquinimod treatment. After immunization with the neuritogenic peptide aa 53–78 of P2 myelin protein, preventive therapy with 12.5 mg/kg laquinimod once daily inhibited neuritis in clinical and electrophysiological terms. Histology corroborated a lower degree of inflammatory lesions and demyelination in the sciatic nerve. The proportion of FoxP3-positive regulatory T cells in the peripheral lymph nodes of treated rats remained unchanged. We conclude that laquinimod may represent a therapeutic option in human autoimmune neuropathies.

1. Introduction

The spectrum of inflammatory demyelinating polyneuropathies ranges from the acute variant, Guillain–Barre syndrome (GBS), to chronic inflammatory demyelinating polyneuropathy (CIDP), which is a progressive or relapsing form of motor and/or sensory dysfunction. The incidence of typical GBS is according to population-based studies in Europe 1.2–1.9 per 100,000, while the prevalence of CIDP is estimated between 2 and 7 per 100,000 (Yoon et al., 2011; Hughes et al., 2013).

Both diseases are pathogenetically characterized by an autoimmune reaction directed against specific components of the peripheral myelin sheath or possibly even the axon (Zhang et al., 2013). In the case of GBS, even under treatment with intravenous immunoglobulins and plasma exchange, mortality rate is up to 4%, and up to 30% of the patients remain permanently disabled. Even for those who recover well, residual weakness and fatigue are common factors of lifelong disability (Kieseier et al., 2004). As a consequence, a better understanding of the underlying immunological mechanisms and new therapeutic options are needed.

The clinical course, electrophysiological characteristics, histological appearance and immunological features of the most common subtype of GBS, the acute inflammatory demyelinating polyradiculoneuropathy (AIDP), resemble the animal model of experimental autoimmune neuritis (EAN), which has immensely contributed to a better understanding of the underlying immune mechanisms (chapter on experimental autoimmune neuritis by Dyck and Thomas, 2005 Peripheral Neuropathy, 4th Edition). Acute EAN, which was first described by Waksman and Adams in 1955, can be induced in rats by the inoculation of susceptible strains with various peripheral nervous system (PNS) antigens, like heterogeneous peripheral nerve myelin, myelin proteins P0 and P2 or peptides with antigenic epitopes emulsified in complete Freund's adjuvant (CFA) (Waksman and Adams, 1955; Kadlubowski and Hughes, 1979). Following immunization, emulsified peripheral myelin antigen is processed by local dendritic cells (DCs) and brought to secondary lymphoid organs, where the activation of naïve peripheral myelin autoantigen-specific lymphocytes takes place. These lymphocytes migrate across the blood–nerve barrier (BNB) via interaction with adhesion molecules such as selectins and integrins, as well as matrix metalloproteinases (e.g., MMP-7 and MMP-9). In the PNS, there is reactivation and amplification of the immune response via pro-inflammatory cytokines and

Abbreviations: GBS, Guillain–Barre syndrome; CIDP, chronic inflammatory demyelinating polyneuropathy; AIDP, acute inflammatory demyelinating polyneuropathy; EAN, experimental autoimmune neuritis; EAE, experimental autoimmune encephalomyelitis; PNS, peripheral nervous system; CFA, complete Freud's adjuvant; DC, dendritic cells; BNB, blood nerve barrier; MMP, matrix metalloproteinases; CNS, central nervous system; VLA-4, very late antigen-4; APC, antigen presenting cells; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; MNC, mononuclear cells; CMAP, compound muscle action potential; MNCV, motor nerve conduction velocity; i.p, intraperitoneally; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; LFA-1, leucocyte function associated antigen-1; pDC, plasmytocytoid dendritic cells.

* Corresponding author at: Dept. of Neurology, Ruhr University, St. Josef-Hospital, Gudrunstr. 56, 44791 Bochum, Germany. Tel.: +49 17662923235; fax: +49 234 5093024.
** Corresponding author. Tel.: +49 234 5092411; fax: +49 234 5092414.
E-mail addresses: kalliapit@yahoo.gr (K. Pitarokoili), ralf.gold@rub.de (R. Gold).
[1] Indicates equal contribution.

http://dx.doi.org/10.1016/j.jneuroim.2014.06.012
0165-5728/

chemokines resulting in the recruitment of macrophages and B-lymphocytes. Macrophages represent the major cell population in inflamed PNS; serve as antigen-presenting and major effector cells of demyelination. Demyelination and axonal damage can occur by multiple mechanisms, including direct phagocytic attack by macrophages, damage from Th1 cytokines and oxygen-free radicals, T-cell- mediated cytotoxicity, complement-dependent attack, and antibody-mediated functional impairment (Mäurer and Gold, 2002). Recovery from EAN coincides with increased interleukin-10 (IL-10) expression and is associated with T-cell apoptosis. Schwann cells can also be active participants either by expressing MHC class II molecules or Fas-Ligand that can induce apoptosis of Fas-Ligand-expressing T cells (Wohlleben et al., 2000).

Laquinimod (ABR-215062) is a new oral immunomodulatory agent successfully evaluated in adult RRMS patients in phase II, phase IIb (LAQ/5062; NCT00349193) and phase III (ALLEGRO, NCT00509145, Comi et al., 2012) clinical studies. Additionally, the BRAVO phase III study (NCT00605215) investigated the effect of laquinimod compared to IFN-beta 1a. Phase III trials in patients with relapsing–remitting multiple sclerosis indicated moderate effects of 0.6 mg oral laquinimod once daily on relapse rate, yet delivered strong evidence for neuroprotection as revealed by slowed disease progression and reduction of brain atrophy as measured by MRI, coupled with a favorable safety and tolerability profile (Filippi et al., 2013, Haggiag et al., 2013).

The exact mechanism of action of laquinimod, when administered orally, is not fully elucidated and is mostly based on studies in EAE. Zou et al. reported that laquinimod applied subcutaneously, ameliorated EAN and induced a shift towards Th2 cytokines (Zou et al., 2002). Based on EAE studies, laquinimod has been suggested to reduce leukocyte migration into the central nervous system (CNS) by down regulation of VLA-4-mediated adhesiveness, inhibiting Th17- proinflammatory responses and also by modulating the cytokine balance in favor of Th2/Th3 (Wegner et al., 2010; Aharoni et al., 2012; Jolivel et al., 2013). In vitro experiments on human B cells from healthy or MS patients have shown that laquinimod modulates B-cell activity, probably by promoting an $IL10^{+}$ $CD86^{+}$ $CD25^{+}$ B-cell subset with regulatory features (Toubi et al., 2012). Furthermore, in laquinimod- treated mice $Foxp3^{+}$ T-regulatory cells were 2.5-fold augmented within the overall inflammatory cells and the T cells in the CNS lesions, respectively, in comparison with untreated mice (Aharoni et al., 2012).

Recently it has been suggested that the beneficial effect of laquinimod may be mediated by modulation of dendritic cells affecting the antigen presentation capacity (Gurevich et al., 2010; Thöne et al., 2012). Treatment with laquinimod of mature DCs results in reduced production of chemokines with a consequent impact on the migratory properties of monocytes (Jolivel et al., 2013). In vivo laquinimod treatment modulates subpopulations of myeloid antigen-presenting cells (APC), including a decrease in DCs and an elevation of anti-inflammatory type II monocytes (Schulze-Topphoff et al., 2012). Several experimental studies converge on the hypothesis that the biological effects of the laquinimod are mainly mediated via inhibition of NF-kB pathway in astrocytes. In this context, it was shown that LAQ prevented cuprizone-induced demyelination and microglial activation in wild type and Rag-1-deficient mice by attenuating astrocytic NF-kB activation. Astrocytic activation can cause deleterious effects by increasing excitotoxicity and hampering neurite outgrowth so that downregulation of this response may constitute an important neuroprotective mechanism (Gurevich et al., 2010; Brück et al., 2012; Mishra et al., 2012; Jolivel et al., 2013).

Evidence is also accumulating that laquinimod might have a neuroprotective effect in EAE and in MS, which is not only due to the effects of immunomodulation, but also to a direct effect of laquinimod in the CNS (Aharoni et al., 2012; Ruffini et al., 2013). Laquinimod treatment induces in situ overexpression of brain-derived neurotrophic factor (BDNF) in the cortex and basal ganglia of chronic EAE mice, as compared with untreated mice. This is associated with significantly reduced myelin and axonal damage and with preservation of the CNS tissue (Aharoni et al., 2012; Thöne et al., 2012). Also in patients with MS, laquinimod treatment results in a significant and persistent increase in BDNF serum levels when compared with baseline and placebo-treated patients (Thöne et al., 2012).

The analogy of the autoimmune mechanisms involved in multiple sclerosis and Guillain–Barre syndrome, and their animal models with the effector role of autoimmune T-cell activation and regulation, has led us to investigate the effect of orally administered laquinimod in EAN and its mechanism of action, with the purpose of providing evidence for a immunomodulatory treatment of autoimmune peripheral neuropathies with laquinimod.

2. Materials and methods

2.1. Antigens

The neuritogenic P2 peptide, corresponding to the amino acids 53–78 of rat myelin P2 protein, was synthesized by Dr. Rudolf Volkmer from Charité University (Berlin, Germany).

2.2. Induction of EAN and assessment of clinical score

A total of 38 female Lewis rats, 6–8 weeks old, purchased from Charles River Co. (Sulzfeld, Germany) and weighing 160–180 g were used in the present study. All animals were anesthetized by exposure to 1.5%–2.0% halothane in oxygen and immunized by subcutaneous injection into the root of the tail of 250 μg $P2_{53-78}$ peptide in PBS, emulsified in an equal volume of complete Freund's adjuvant (CFA) containing *Mycobacterium tuberculosis* H37RA (Difco, Detroit, MI). Animals were weighed and scored for disease severity daily by two investigators. Disease severity was assessed clinically employing a scale ranging from zero to 10 originally described by Enders et al. (1998): 0 normal; 1 less lively; 2 impaired righting/limb tail; 3 absent righting; 4 atactic gait, abnormal position; 5 mild paraparesis; 6 moderate paraparesis; 7 severe paraplegia; 8 tetraparesis; 9 moribund; 10 death. All experiments were reviewed and approved by the North-Rhine-Westphalia authorities for animal experimentation (TVA 84–02.04.2014-A106).

2.3. In vivo treatment with laquinimod

Laquinimod (ABR-215062) (TEVA Pharmaceutical Companies) was dissolved in tap water and a total volume of 300 μl was administered by a daily oral gavage starting from the day of immunization to day 28 p.i; control groups received similar volume of water. Rats were given ad libitum access to food and water and were housed at the animal facility of the Ruhr-University Bochum. The animals were randomly divided into the following groups: control group treated with tap water ($n = 11$), a 6.25 mg/kg body weight laquinimod-treated group ($n = 5$), a 12.5 mg/kg body weight laquinimod-treated group ($n = 11$) and a 25 mg/kg body weight laquinimod-treated group ($n = 11$). The experiments were repeated twice.

2.4. Electrophysiological analysis

Nerve conduction tests were performed on day before immunization (-1) and on days 16 (maximum of natural disease course) and 27 (recovery) post-immunization. At each time point 11 rats per group were tested for sham-treated rats, 12.5 mg/kg and 25 mg/kg laquinimod-treated group and 5 rats for 6.25 mg/kg laquinimod-treated group. The rats were anesthetized intraperitoneally (i.p.) with xylazine and ketamine (10 mg/kg and 50 mg/kg respectively). By examining amplitude and latencies of the evoked compound muscle action potentials (CMAPs) recorded from the feet, we assessed sciatic nerve motor conduction. Using a fully digital recording Keypoint apparatus (Dantec, Skovlunde, Denmark) and paired needle electrodes inserted at the sciatic notch (hip; proximal) or the popliteal fossa (distal), the sciatic nerve was