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Oral treatment with laquinimod augments regulatory T-cells and brain-derived neurotrophic factor expression and reduces injury in the CNS of mice with experimental autoimmune encephalomyelitis

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ABSTRACT

Laquinimod is an orally active molecule that showed efficacy in clinical trials in multiple sclerosis. We studied its effects in the CNS, when administered by therapeutic regimen to mice inflicted with experimental autoimmune encephalomyelitis (EAE). Laquinimod reduced clinical and inflammatory manifestations and elevated the prevalence of T-regulatory cells in the brain. In untreated mice, in the chronic disease stage, brain derived neurotrophic factor (BDNF) expression was impaired. Laquinimod treatment restored BDNF expression to its level in healthy controls. Furthermore, CNS injury, manifested by astrogliosis, demyelination and axonal damages, was significantly reduced following laquinimod treatment, indicating its immunomodulatory and neuroprotective activity.

1. Introduction

Laquinimod (quiniline-3-carboxamide) is a novel orally immunomodulatory drug developed for the treatment of relapsing remitting multiple sclerosis (RRMS). Clinically, laquinimod induced beneficial effects and reduced gadolinium-enhancing lesions in relapsing remitting MS (RRMS) (Comi et al., 2008, 2012) and in the animal model of MS–experimental autoimmune encephalomyelitis (EAE) (Brunmark et al., 2002). In both these pathologies autoreactive immune cells infiltrate into the CNS, triggering within it inflammatory mechanisms such as resident microglial and astrocyte activation (Hellings et al., 2002; Hohlfeld and Wekerle, 2004; Behi et al., 2005). The autoimmune reactivity against the myelin constituents leads to its destruction with subsequent widespread axonal damage. In addition, increasing evidence indicates that MS and EAE are complex disorders that involve axonal and neuronal pathology in the gray as well as in the white matter (Lassmann et al., 2007; Bruck, 2008; Trap and Nave, 2008).

Subsequent to the pathological process, immunomodulatory and neuroprotective routes are stimulated in MS/EAE to counteract and restore the damage (Aharoni and Arnon, 2009). T-regulatory cells (Tregs), which are potent immunosuppressors that ameliorate a wide array of inflammatory conditions, were shown to modulate MS and EAE (Vila et al., 2009). Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) are essential for the development and the maintenance of the CNS by mediating axonal growth, neuronal activity, synaptic and dendritic plasticity, via receptor tyrosine kinase (Kalb, 2005). In pathological conditions BDNF expression was shown to correlate with reduced neuronal death, and promoted axonal outgrowth, remyelination and regeneration. Impaired BDNF expression is implicated in neuronal death and degeneration (Murer et al., 2001; Linker et al., 2010). Neurons are the main source of BDNF in the CNS, but other cells such as activated astrocytes and infiltrating T-cells, present in inflamed sites, are also a potential source (Murer et al., 2001; Riley et al., 2004). Increased BDNF levels at the early phases of EAE and MS reflect self-repair neuroprotective mechanisms, however, as the disease progresses, the levels of neurotrophic factors decline. Notably, several studies demonstrated that BDNF secretion may be elevated and restored by immunomodulatory treatment such as glatiramer acetate (Aharoni et al., 2005a; Azoulay et al., 2005).

Previous studies have indicated that the mechanism of action of laquinimod involves immunomodulation. Thus, laquinimod down-regulated migration of leukocytes into the CNS and reduced the Th1 as well as Th17 proinflammatory responses, in the EAE model (Yang et al., 2004; Wegner et al., 2010; Brück and Wegner, 2011). The decrease in inflammation following laquinimod treatment was accompanied by

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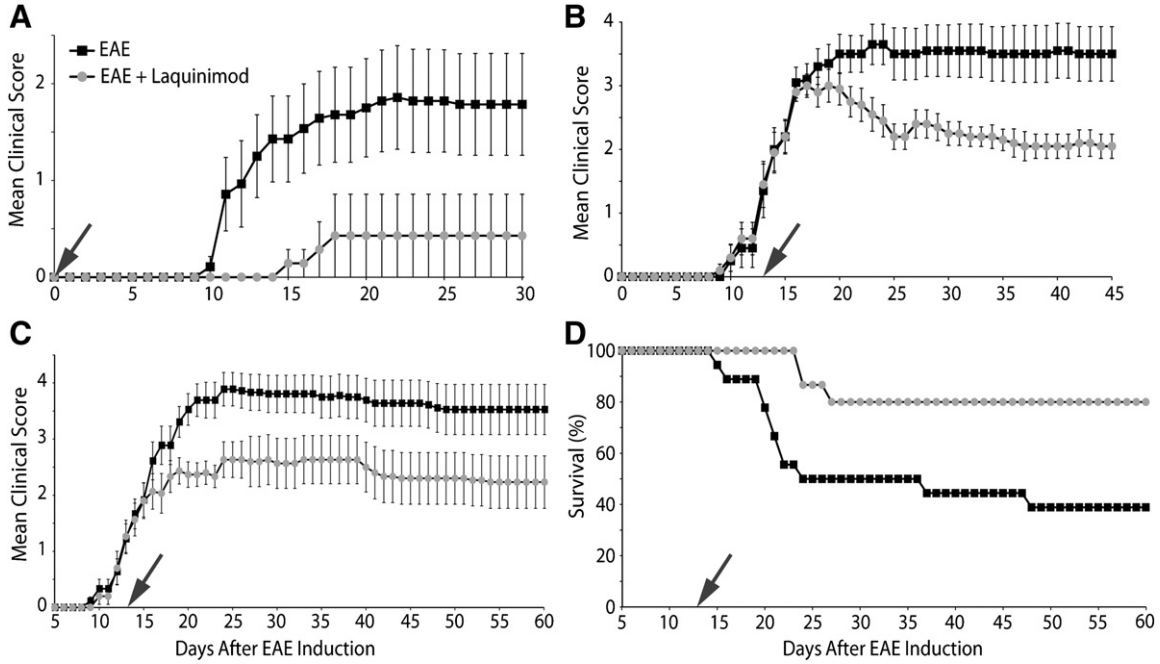

Fig. 1. The effect of laquinimod on clinical manifestations of MOG-induced EAE. A. Laquinimod prevention treatment initiated on the day of disease induction (N = 7), in comparison to water treatment (N = 14). B. Laquinimod suppression treatment initiated 15 days after disease induction (N = 10), in comparison to water treatment applied to EAE-mice with identical disease scores (N = 10). C. Laquinimod suppression treatment initiated 13 days after disease induction (N = 15), in comparison to water treatment applied to EAE-mice with identical disease score (N = 18). In A–C the mean clinical scores with their standard errors are depicted. D. Survival curve — the effect of laquinimod suppression treatment initiated 13 days after disease induction (N = 15), in comparison to water treatment applied to mice with identical disease score (N = 18). Laquinimod was administered orally, 0.25 mg/mouse, daily till the end of the experiment (the day of treatment initiation is depicted by an arrow). Scores of 5 were assigned to euthanized or dead animals and carried on till the end of the experiments. Representative experiments from 10 experiments performed.

reduced myelin and axonal damage (Wegner et al., 2010). In a recent study, increased BDNF levels were found in blood samples of MS patients treated with laquinimod (Thöne et al., 2012). Furthermore, laquinimod was less effective in EAE-induced knockout mice lacking BDNF in their myeloid cells and T-cells, suggesting BDNF involvement in the beneficial effect induced by this treatment.

In the current study we further investigated several aspects of laquinimod activity, mainly its immunomodulatory and neuroprotective effects in the CNS. In particular we were interested in characterizing the consequences involved in laquinimod therapeutic activity in situ when applied after disease exacerbation. We report herewith that laquinimod treatment of EAE inflicted mice resulted in reduced inflammation with paralleled elevation in T-regulatory cells in the brain. Furthermore, in treated mice BDNF expression was restored to its level in healthy controls and astrogliosis as well as CNS damages were reduced, supporting immunomodulatory and neuroprotective mechanisms induced by this drug.

2. Materials and methods

2.1. Animals

C57BL/6 mice were purchased from Harlan (Jerusalem, Israel). Yellow fluorescent protein (YFP) mice (originated from C57BL/6 and CBA hybrids) which selectively express YFP in their nervous system (in the neural cytoplasm), under the Thy1 promoter (Feng et al., 2000), were kindly provided by Joshua R. Sanes (Washington University, St. Louis, MO). Female mice, 8–12 weeks of age, were kept under specific pathogen free (SPF) environment. All experiments were approved by the Institutional Animal Care and Use Committee of the Weizmann Institute.

2.2. EAE

EAE was induced in C57BL/6 mice by the peptide encompassing amino acids 35–55 of myelin oligodendrocyte glycoprotein (MOG), synthesized by Genscript (Piscataway, NJ). Mice were injected subcutaneously at the flank, with 200 µl emulsion containing 300 µg of the encephalitogenic peptide in incomplete Freund's adjuvant enriched with 3.5 mg/ml heat-inactivated *Mycobacterium tuberculosis* (Sigma). Pertussis toxin (Sigma), 200 µg/mouse was injected intraperitoneally immediately after the encephalitogenic injection and 48 h later. Mice were examined daily. EAE was scored as follows: 0—no disease, 1—limp tail, 2—hind limb paralysis, 3—paralysis of all four limbs, 4—moribund condition, and 5—death. Scores of 5 were assigned to euthanized or dead animals and carried on till the end of the experiments.

2.3. Laquinimod

Laquinimod (originally ABR-215062) (RLB#054 M0004) was synthesized by Teva Pharmaceutical Industries, Ltd. The compound was dissolved in purified water and administered orally by gavage, 25 mg/kg, daily, in a volume of 0.2 ml. Treatment was initiated either immediately following EAE induction (prevention regimen) or after the development of clinical manifestations (suppression regimen), until the end of the experiment. Control EAE mice were similarly fed by 0.2 ml water (EAE untreated).

2.4. Perfusion and organ processing

Mice were deeply anesthetized and perfused transcardially with 2.5% paraformaldehyde. Brains and spinal cords were dissected and postfixed (48 h, 4 °C). Organs were either paraffin embedded and