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Sphingosine 1-phosphate receptor modulator fingolimod (FTY720) does not promote remyelination in vivo☆

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ABSTRACT

The sphingosine 1-phosphate (S1P) receptor modulators have emerged as a new therapeutic opportunity paradigm for the treatment of immune-mediated demyelinating diseases such as multiple sclerosis (MS). The S1P analog fingolimod (FTY720) has been shown to alleviate disease burden in immune-mediated animal models of MS, and has been approved for treatment in clinical trials in patients with MS in the United States. While the immunological effects of FTY720 are well established, there is controversy in the literature regarding the contribution of FTY720 on myelin repair. Here, we directly assessed the impact of FTY720 on myelin repair in cuprizone and lysolecithin (LPC) demyelination models that have a minimal immunological component. FTY720 failed to promote remyelination in either animal model. These studies suggest that while FTY720 may be effective at modulating the immunological attack in MS, it may benefit from an add-on therapy to enhance the myelin repair required for long-term functional restoration in MS.

Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating disease characterized by areas of demyelination and loss of oligodendrocytes (Compston and Coles, 2008). A major effector of this central nervous system (CNS) damage is thought to be T cell infiltration across the blood brain barrier. These T cells target CNS antigens to damage myelin, resulting in demyelinated lesions throughout the brain and spinal cord (Compston and Coles, 2008). The subsequent inability of CNS processes to remyelinate these damaged lesions results in neurodegeneration and various cognitive and physical neurological deficits. As a result, effective functional restoration in MS may depend on the ability to remyelinate and thereby regenerate healthy axons and neurons.

Any treatment effect on myelin repair is likely to affect oligodendrocyte precursor cell (OPC) maturation. Myelination is known to result from directed activation of complex cellular processes, including OPC proliferation, migration, adhesion, process extension/retraction, and differentiation (Miller and Mi, 2007; Miron et al., 2008b). Furthermore, detailed functional studies of repair can be accomplished through the use of a range of approaches, including the analysis of oligodendrocyte differentiation in vitro and the use of ex vivo brain slice cultures that permit cellular and molecular perturbation of the environment of actively myelinating systems. In previous chemical-induced demyelination studies, we have used these approaches to demonstrate that inhibition of leucine-rich repeat- and Ig domain-containing Nogo receptor-interacting protein 1 (LINGO-1) function promotes myelin formation that is correlated with promotion of OPC differentiation and maturation of oligodendrocytes (Mi et al., 2007; Mi et al., 2009).

Sphingosine 1-phosphate (S1P) is a signaling sphingolipid that mediates a variety of cellular responses through the endothelial differentiation G-protein coupled (EDG) receptors. S1P signaling plays a critical role in immunomodulation and has recently become a focus of attention in the treatment of immune-mediated demyelinating diseases such as MS (Brinkmann et al., 2010). One modulator of this system is the S1P analog fingolimod (FTY720), a non-specific agonist for S1P receptors that possesses clear anti-inflammatory properties (Brinkmann et al., 2010). FTY720 (Gilenya) has recently been approved as an oral agent for use in relapsing forms of MS in the United States (Cohen and Rieckmann, 2007; Hemmer and Hartung, 2007). Clinical studies have shown that Gilenya treatment reduces annual relapse rate and severity of symptoms (Cohen et al., 2010; Kappos et al., 2006, 2010; O'Connor et al., 2009). The primary mechanism of FTY720 efficacy is believed to be immunological, whereby FTY720 prevents lymphocyte egress from lymphoid organs, thus inhibiting the circulation of autoaggressive T cells (Compston and Coles, 2008).

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Experimental autoimmune encephalitis (EAE) is an animal model for studying inflammatory diseases of the central nervous system and has contributed valuable insights into the effects of immunologically-induced demyelination at the whole organism level (Furlan et al., 2009). FTY720 treatment has been shown to ameliorate symptoms of EAE disease and some studies even report evidence of enhanced myelination (Foster et al., 2007; Kataoka et al., 2005; Papadopoulos et al., 2010). However, it is unclear whether the increased myelination results from FTY720 indirectly inhibiting demyelination by blocking T cell infiltration into the CNS or from FTY720 directly promoting OPC maturation and remyelination. There are limited *in vivo* studies that directly address the role of FTY720 on OPC proliferation, differentiation and remyelination, which could be critical for understanding the long term effects of FTY720 on CNS remyelination and repair. A non-immune-based animal model, one where the primary demyelination is not mediated by immunological attack, is necessary in order to determine whether FTY720 treatment promotes remyelination. In this study, we used two such models, cuprizone and lysolecithin (LPC), to study the specific effects of FTY720 on remyelination. These models allow clearer temporal delineation of the phases of demyelination and remyelination in the absence of primary involvement of the immune system. Our studies demonstrate that FTY720 does not promote remyelination in either model.

Results

Local delivery of FTY720 induces demyelination in the LPC model

The lysolecithin-induced (LPC) demyelination model was utilized to assess the effects of FTY720 on remyelination *in vivo*. LPC was injected into rat dorsal columns to produce demyelinated lesions after a few hours. 3 days after LPC injection, animals were dosed with FTY720, anti-LINGO-1 antibody, or vehicle control treatment by direct injection into the lesion. Remyelination was examined on day 10 post-LPC injection by luxol fast blue (LFB) staining (Fig. 1A, top panels). FTY720 treatment led to a two-fold increase in the size of demyelinated lesions compared to the vehicle control treatment group, as determined by 3-dimensional measurement (Fig. 1B; control $n=5$, FTY720 $n=7$). In contrast, anti-LINGO-1 antibody treatment reduced the lesion size 18-fold compared with controls (Fig. 1A, B; $n=8$), consistent with our previous finding that inhibiting LINGO-1 promotes remyelination (Mi et al., 2009). The FTY720-treated animals contained non-LFB staining spots in the white matter perilesional areas with moth-eaten morphology (Bradl and Lassmann, 2010) characteristic of OPC death and demyelination (Fig. 1C, D). This morphology suggests that locally delivered FTY720 may diffuse into nearby white matter tissue to induce OPC death and demyelination. To clarify the direct effect of FTY720 on demyelination, FTY720 was

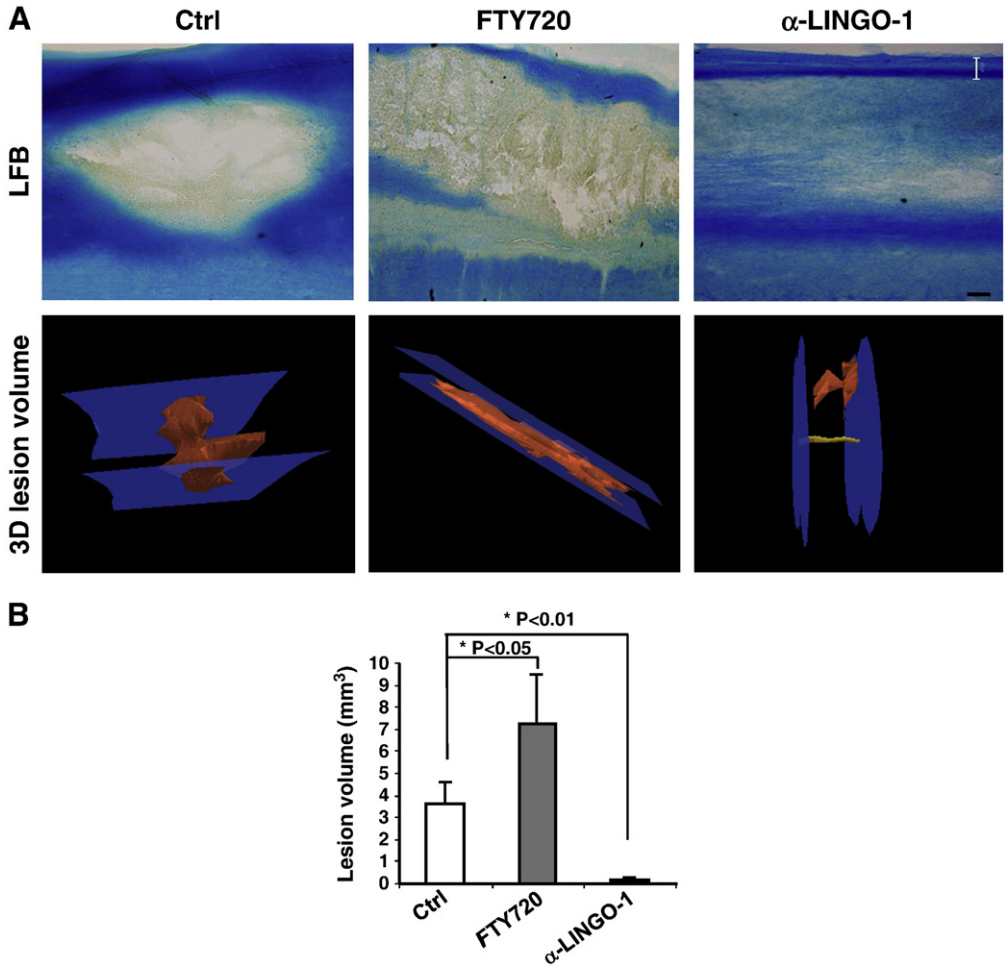

Fig. 1. Local delivery of FTY720 causes demyelination in the LPC model. (A) Luxol fast blue staining (LFB) of white matter in spinal cord dorsal column lesions following local injection of FTY720, vehicle control, or anti-LINGO-1 antibody (top panels). Bottom panels show three-dimensional reconstructions of the lesion areas (red, lesion volume). Scale bar = 100 μm. (B) Quantification of the lesion volumes of (A, bottom panels). (C) LFB staining of spinal cord tissue surrounding lesions from vehicle control- and FTY720-treated rats. Arrows denote moth-eaten morphology. (D) Enlargement of right panel from (C). (E) Black gold staining of brain slice cultures to visualize demyelination by FTY720P treatment. (F) Quantification of black gold staining intensity in (E).