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ENHANCED REMYELINATION FOLLOWING LYSOLECITHIN-INDUCED DEMYELINATION IN MICE UNDER TREATMENT WITH FINGOLIMOD (FTY720)

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Abstract—Multiple sclerosis (MS) is a chronic, progressive demyelinating disorder which affects the central nervous system (CNS) and is recognized as the major cause of nervous system disability in young adults. Enhancing myelin repair by stimulating endogenous progenitors is a main goal in efforts for MS treatment. Fingolimod (FTY720) which is administrated as an oral medicine for relapsing-remitting MS has direct effects on neural cells. In this study, we hypothesized if daily treatment with FTY720 enhances endogenous myelin repair in a model of local demyelination induced by lysolecithin (LPC). We examined the response of inflammatory cells as well as resident OPCs and evaluated the number of newly produced myelinating cells in animals which were under daily treatment with FTY720. FTY720 at doses 0.3 and 1 mg/kg decreased the inflammation score at the site of LPC injection and decreased the extent of demyelination. FTY720 especially at the lower dose increased the number of remyelinated axons and newly produced myelinating cells. These data indicate that repetitive treatment with FTY720, behind an anti-inflammatory effect, exerts beneficial effects on the process of endogenous repair of demyelinating insults.

Key words: fingolimod (FTY720), lysolecithin, myelin repair, oligodendrocyte progenitors, mouse.

INTRODUCTION

Multiple sclerosis (MS) is the most common autoimmune demyelinating disease (Ransohoff, 2012), characterized by inflammatory infiltration of immune cells into the central nervous system (CNS) which leads to oligodendrocyte death followed by demyelination and axon degeneration (Frohman et al., 2006). MS causes physical and cognitive disability and interferes with lifestyle. Following demyelination, endogenous progenitors and stem cells of adult CNS contribute to myelin repair which may explain for recovery from MS attacks. An effective remyelination depends on proliferation of immature oligodendrocyte progenitors (OPCs), their migration to the site of injury, and differentiation into myelinating oligodendrocytes (Peterson and Fujinami, 2007). Inflammatory process contributes to recruitment of neural stem cells and OPCs (Ben-Hur et al., 2003, Imitola et al., 2004). The majority of available therapies for MS inhibit inflammation to slow down the progress of disease and to rescue from inflammatory attack. Therefore a major concern on the application of immune system modulator drugs is their effect on the process of endogenous remyelination. An ideal treatment of MS, should inhibit inflammation by targeting the immune system and enhance myelin repair by recruiting resident endogenous OPCs as well as by protecting oligodendrocyte lineage cells (Hemmer and Hartung, 2007).

Fingolimod (FTY720), a sphingosine 1-phosphate (S1P) receptor modulator, was the first oral medicine for treating MS which was approved in 2010 (Al-Izki et al., 2011; Pelletier and Hafler, 2012). FTY720 administration in relapsing–remitting MS patients improved the rate of relapsing and disease activity as measured by MRI (Kappos et al., 2010). FTY720 may exert both immunological and non-immunological mechanisms of action (Brinkmann et al., 2010; Choi et al., 2011; Chun and Brinkmann, 2011; Cohen and Chun, 2011). Through its immunological effect, FTY720 prevents lymphocytes egress from the lymph nodes into the blood and therefore inhibits their consequent infiltration into the CNS (Pinschewer et al., 2000; Mehling et al., 2008; Gasperini and Ruggieri, 2012). In MS patients, FTY720 reduced the number of naive and memory T cells in the blood (Mehling et al., 2008). Rau and colleagues also showed the lower rate of infiltration of macrophages and microglia following FTY720 treatment (Rau et al., 2011).

There is some evidence for direct effect of FTY720 on the nervous system. FTY720 crosses the blood–brain barrier because of its lipophilic structure, and S1P receptors are expressed by different cell types of CNS such as OPCs (Soliven et al., 2011; Groves et al., 2013), oligodendrocytes (Jaillard et al., 2005), neurons (Kimura et al., 2007) and astrocytes (Van Doorn et al., 2010) under

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Abbreviations: EAE, experimental autoimmune encephalomyelitis; H&E, hematoxylin and eosin; LFB, Luxol Fast Blue; LPC, lysolecithin; MS, multiple sclerosis; OPCs, oligodendrocyte progenitor cells; S1P, sphingosine 1-phosphate.

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both physiological and pathological conditions (Chun et al., 2000). This has encouraged the researchers to evaluate direct and non-immunological effects of FYT720 on CNS (Miron et al., 2010; Kipp and Amor, 2012).

Experimental data have demonstrated that FTY720 reduces demyelination, restores or preserves neuronal cell function. FTY720 sustained or promoted nerve function in MOG-induced encephalomyelitis (EAE) by promoting endogenous repair mechanisms (Balatoni et al., 2007). In another study using conditional knockout mice lacking S1P1 in the astrocytes, FTY720 did not attenuate the EAE scores (Choi et al., 2011). Additionally, FTY720 promoted the survival of oligodendroglial lineage cells and enhanced synaptic functions (Jung et al., 2006; Balatoni et al., 2007; Choi et al., 2011; Rossi et al., 2012). Ex vivo studies using organotypic culture of brain slices showed that FTY720 directly acts on OPCs and enhanced their differentiation to myelinating oligodendrocytes following lysolecithin (LPC)-induced demyelination (Miron et al., 2010). Therapeutic administration of FTY720 following LPC injection into the rat dorsal column led to a significant increase in the size of demyelinated lesion and OPCs death; and its administration on rat brain slices reduced myelination in the corpus callosum (Hu et al., 2011). In cuprizone model of demyelination, FTY720 treatment modestly accelerated myelin recovery after acute demyelination (Slowik et al., 2015). In EAE as an inflammatory animal model of MS, FTY720 promoted proliferation and differentiation of OPCs (Zhang et al., 2015). Altogether, the effects of FTY720 on OPCs proliferation and differentiation seem to be not fully understood. Moreover, it would be crucial to elucidate the characteristics of a demyelinating attack in MS patients, which are daily exposed to the prophylactic administration of FTY720.

In the current study, we challenged mice which were under daily treatment of FTY720 by a local injection of LPC as a toxin which induces local demyelination. Afterward, we studied the intensity of the inflammatory response at the lesion site and the response of resident OPCs and their contribution into remyelination.

EXPERIMENTAL PROCEDURES

Animals

Eight- to ten-week-old (20–25 g), C57BL/6 male mice were purchased from Pasteur Institute (Karaj, Iran) and maintained in groups of four per cage under 12-h light/12-h dark cycles. All experiments were conducted in compliance with the NIH guidelines for the care and use of laboratory animals. Procedures were approved by Tarbiat Modares University Committee of Ethics in Research. Efforts were made to minimize the animals' suffering and to reduce the number of animals used.

Interventions

Animals were daily gavaged by FTY720 (Danesh Pharmaceutical Development Co., Tehran, Iran). Oral dose of 0.3 or 1 mg/kg FTY720 was selected based on the literature (Kataoka et al., 2005; Al-Izki et al., 2011; Kim et al., 2011; Zhang et al., 2015). FTY720 administration was continued till the time of sacrificing of animals. In all experimental groups except the intact, under anesthesia, a direct injection of 1 μL of 1%) w/v) LPC (Hu et al., 2011) into the corpus callosum (AP: 1.1 mm and L: 1.2 mm from the Bregma, DV: 2.2 mm from the dura; (Paxinos and Franklin, 2004)) was done following 6 days administration of FTY720. For inducing anesthesia mice received ketamine (70 mg/kg, i.p., Alfasan, Holland) and xylazine (10 mg/kg, i.p.).

Mice were enrolled into the following groups: (1) animals which received no treatment (intact); (2 and 3) animals which received FTY720 (0.3 or 1 mg/kg/day, P. O.), for eight days and were sacrificed at day 9; (4) animals which received tap water for 8 days and were sacrificed at day 9 as the control group; (5 and 6) animals treated with FTY720 (0.3 or 1 mg/kg, P.O.) for 12 days; (7) animals which received tap water for 12 days. When required, bromodeoxyuridine (BrdU, Sigma–Aldrich) 100 mg/kg, an analogous of thymine, was administrated intraperitoneally (i.p.) for 6 days to label the proliferating cells. BrdU injection was started at the time of LPC injection (Zhang et al., 2015).

Immunohistofluorescence

At the time of sacrificing, animals were re-anesthetized by chloral hydrate (480 mg/kg), then transcardially perfused with PBS followed by 4% paraformaldehyde. The brains were removed, post-fixed in 4% paraformaldehyde for overnight, and sectioned as 6-μm coronal sections using a cryostat device (Histo-Line Laboratories, Milan, Italy). Sections from area between Bregma +1.3 to Bregma 0.9 mm (Paxinos and Franklin, 2004) were collected for the following studies.

Immunostaining was performed as previously described (Dehghan et al., 2015). Briefly sections were incubated in triton-X100, blocked using normal goat serum, and then were incubated overnight in primary antibody at 4 °C. Staining against Olig2 (Fancy et al., 2004) and CD45 (Wang et al., 2011) were employed to identify OPCs and total infiltrated leukocytes, respectively. Antibody against PLP was used to demonstrate tissue myelination. BrdU/Olig2 double staining was performed to detect newly produced oligodendrocyte lineage cells. After washing with PBS, sections were incubated with secondary fluorescence-labeled antibody for 1 h at room temperature. The characteristics of all applied primary and secondary antibodies are presented in Table 1. The sections were finally mounted using UltraCruz™ mounting medium containing DAPI (Santa Cruz Biotechnology Inc., CA, USA, sc-24941) and evaluated using BX51 Olympus microscope. Images were captured using an Olympus DP72 camera for consequent analysis. For quantification of immunostained cells, three sections per animal were stained and the counts were averaged. Then data entered into final averaging for three animals per experimental group.

Hematoxylin and eosin staining

A series of 6-μm-thick coronal sections were prepared from paraffin-embedded brain samples, then 21 sections