Commentary

The Impact of Fingolimod (FTY720) in Neuroimmunologic Diseases

Mechanisms Beyond Immunomodulation

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A line of novel drugs developed for the treatment of relapsing remitting multiple sclerosis (MS) has emerged, some of which have just passed controlled Phase III clinical trials. These therapeutics share a common feature: the prospect of better tolerability and oral administration, in comparison with the currently approved MS therapeutics that have been in use for almost two decades. Some of these new drugs promise superior efficacy compared with injectable β interferon preparations or glatiramer acetate as well.

Just recently, the results of two extensive, controlled clinical studies evaluating the efficacy and safety of oral fingolimod (FTY720) in relapsing remitting multiple sclerosis were reported. In the FREEDOMS study, fingolimod was compared with placebo, and in the TRANSFORMS study, fingolimod was examined in a head-to-head approach with the intramuscularly injected interferon β -1a.^{1,2} In both studies, fingolimod significantly reduced the number of relapses and amount of disease activity in the central nervous system (CNS) by at least 50% compared with the control arm. These clinical data were corroborated by magnetic resonance imaging. However, increased alertness is required in handling this promising drug, as two cases with fatal disseminated herpes simplex and varicella zoster encephalitis and serious cardiac adverse events occurred in the TRANSFORMS study at doses higher than the 0.5 mg/day dose submitted to the FDA and the European Medicines Agency.

Like with other experimental drugs that have been successfully used for the treatment of MS, the full extent of possible beneficial and detrimental mechanisms exerted by fingolimod is incompletely understood. So far, the immunomodulatory facet of fingolimod, namely the impaired egress of immune effector cells out of lymphoid organs resulting in subsequent (mild) lymphopenia, has been proposed to primarily account for its efficacy in

MS.³ In this issue, the group of J. Antel sheds further light on a putative and, to date, somewhat neglected nonimmune mechanism of fingolimod. Their data imply a regenerative and possibly neuroprotective effect within the CNS as fingolimod passes the blood-brain barrier and attains considerable tissue concentrations. Indeed, fingolimod may promote process extension by oligodendrocyte progenitor cells (OPCs) after toxic demyelination, remyelinating axons.⁴

In view of the established downstream actions of sphingosine-1-phosphate (S1P), which are imitated by fingolimod, it is not surprising that the therapeutic response achieved by fingolimod in MS patients might extend beyond immunomodulation. S1P is synthesized by sphingosine kinases 1 and 2 in virtually every cell type through phosphorylation of sphingosine, a plasma membrane-bound sphingolipid. S1P then exerts its effects via five variably expressed G-protein-coupled receptors, S1P1-5.⁵

A variety of immunological properties, among them migration of immune effector cells, cell differentiation, and effector responses assigned to the diversity of S1P receptors, have raised great interest for S1P in the area of autoimmune diseases. In particular, the evidence for a strong inhibitory effect of S1P1-agonism on egress of lymphocytes after their passage through secondary lymphoid organs, such as spleen and lymph nodes, has led to the conclusion that the predominant pharmacomechanism of fingolimod is based on preventing autoaggressive immune cells from accessing their autoantigens.⁶ Indeed, this has been shown to hold true for the therapeutic use of fingolimod in the animal model of experimental autoimmune encephalomyelitis, which reflects many aspects of MS, as well as in experimental autoimmune neuritis, a model for inflammatory demyelinating polyradiculoneuropathies. In these models, oral gavage

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of fingolimod led to a significant amelioration or even prevention of the respective clinical phenotypes,^{7,8} and the induction of lymphopenia correlated with the reduction or even absence of disease activity. However, in neither work was the recovery of oligodendroglial and Schwann cells or the extent of remyelination the focus of the study.

Previously, Jaillard et al had provided evidence for the constitutive expression of S1P5 and its impact on cell differentiation and survival throughout the different oligodendroglial developmental stages. In primary rodent cultures, some variability existed depending on cellular maturity. Whereas S1P led to S1P5-dependent retraction of cell processes in premature oligodendrocytes, it rather promoted survival of differentiated oligodendrocytes.⁹ In addition, Miron et al have demonstrated in primary cultures dissociated from human fetal CNS that OPCs and mature oligodendrocytes show stage-specific expression of S1P1, S1P3, and S1P5, but not S1P4, on the transcriptional level.^{10,11} Furthermore, S1P receptor expression and subsequent oligodendrocytic properties toward myelination, maturation, and survival demonstrated a dose and exposure time-dependent response to fingolimod. According to these data, mature oligodendrocytes in culture extend processes at prolonged exposure to low doses of fingolimod, predominantly by a S1P5- and, to a lesser extent, by S1P3- and S1P1-dependent pathway involving Rho-mediated cytoskeletal elements. However, in OPCs only prolonged fingolimod treatment was shown to have similar effects in terms of membrane elaboration, and these effects were mediated by S1P1.

In the current issue of *The American Journal of Pathology*, Miron et al pursue previous experiments in which they further elucidate the role of S1P on oligodendrocytic maturation, membrane dynamics, and survival.⁴ In a morphologically well-illustrated *in vitro* model of lysolecithin-induced demyelination in organotypic cerebellar slice cultures, which apparently affects only the myelin sheaths and spares the axons from damage, they investigate the role of S1P and its receptors on the capacity of OPCs to perform remyelination. The model used for this study mimics anatomical conditions as observed *in situ* in the CNS involving the major cellular components and their interaction: oligodendrocytes (and their progenitor cells), astrocytes, microglia, and neurons.^{4,12} At the same time, this model allows the circumvention of the peripheral immune system, which is a major target tissue of S1P. Moreover, organotypic cerebellar slice cultures have been suggested to be particularly suitable for visualizing oligodendrocytic dynamics and myelination processes in the CNS, eg, by viral-mediated gene delivery of membrane fluorescent proteins.^{13,14}

Here, the interaction between neurons and oligodendrocytic processes during axonal ensheathment, demyelination after lysolecithin exposure, and remyelination on fingolimod treatment are visualized by confocal, light, and electron microscopy at a high technical level. Miron et al show that fingolimod leads to process extension in mature oligodendrocytes as well as in OPCs, independent of the concentrations chosen. In addition, a reduction of astrocytosis and the number of microglia cells in

control slice cultures was detected at low doses. This is an interesting observation considering that mature oligodendrocytes are currently believed to be relatively stable and to reveal poor plasticity.¹⁴ Indeed, to a certain extent, remyelination as evaluated by myelin basic protein staining was a sign of spontaneous recovery. In addition, fingolimod treatment promoted significant and time-dependent oligodendrocytic process extensions accompanied by functional axonal re-ensheathment, as evaluated by the appearance of myelin at the node of Ranvier in electron microscopic images. Furthermore, fingolimod maintained survival of OPCs over a longer time period. Miron et al also further clarified the role of the differential oligodendrocytic S1P receptors by the engagement of specific S1P1 and S1P3/5 agonists and respective antagonists in the organotypic slice culture model compared with fingolimod. These results suggest a predominant role of oligodendrocytic S1P3/5 in the effects exerted by fingolimod and are in line with their previous findings.

The insights on the role of S1P and its receptors in the dynamics of oligodendrocytes and myelination obtained by Miron et al impressively demonstrate the unforeseen potential of fingolimod, a drug that is about to be approved for the therapy of relapsing remitting MS and under evaluation for progressive forms of the disease. The necessity of using a model that rules out the participation of the immune system to better understand the role of oligodendrocytes, as performed in the discussed work, appears to be feasible. However, the question of how and whether fingolimod influences remyelination in autoimmune-mediated myelin damage in a comparable fashion remains unanswered at the present time point. *In vivo* and *in vitro* models have been assessed to show that the degree of remyelination is modulated by various agents, eg, impaired by statins or enhanced by progesterone.^{15,16} Whether fingolimod is able to promote tissue regeneration in autoimmune disease in MS patients awaits further investigation and might soon be detectable by new techniques such as magnetization transfer magnetic resonance imaging.

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