Sphingosine 1-phosphate receptor modulator, fingolimod (FTY720), provides a new therapeutic approach for autoimmune diseases

Kenji Chiba*, Hirotoshi Kataoka, Yasuhiro Maeda, Noriyasu Seki, and Kunio Sugahara

Pharmacology Research Laboratories I, Research Division, Mitsubishi Tanabe Pharma Corporation, Yokohama, Japan

FTY720 (Fingolimod) is the first of a new immunomodulator class: sphingosine 1-phosphate (S1P) receptor modulator. We have found FTY720 by chemical modification of a natural product, myriocin derived from Isaria sinclairii, a kind of vegetative wasp. FTY720 is orally active and is highly effective in various autoimmune disease models including experimental autoimmune encephalomyelitis (EAE), adjuvant- or collagen-induced arthritis, and lupus nephritis. Particularly, oral administration of FTY720 shows marked therapeutic effects on EAE in mice with a significant reduction of demyelination and T cell infiltration in the central nervous system. A most striking feature of FTY720 is the induction of a marked decrease in peripheral blood lymphocytes at doses that show immunomodulating effects. It is revealed that the reduction of circulating lymphocytes by FTY720 is due to sequestration of lymphocytes into secondary lymphoid organs. FTY720 is rapidly converted to FTY720-phosphate (FTY720-P) by sphingosine kinases. FTY720-P acts as a potent agonist at S1P receptor type 1 (S1P₁), internalizes S1P₁ on lymphocytes, and inhibits migration of lymphocytes toward S1P. It is highly likely that immunomodulating effects of FTY720 are caused by inhibition of S1P/S1P₁-dependent lymphocyte egress from secondary lymphoid organs. Moreover, it is suggested that direct effects of FTY720-P on neural cells via S1P receptors promote neuroprotection. Since FTY720 possesses a novel mechanism of action and is highly effective in relapsing remitting multiple sclerosis patients, it is presumed that oral FTY720 provides a new therapeutic approach for autoimmune diseases including multiple sclerosis.


*Correspondence should be addressed to:
Kenji Chiba, Ph.D., Pharmacology Research Laboratories I, Research Division, Mitsubishi Tanabe Pharma Corporation, 1000, Kamoshida-cho, Aoba-ku, Yokohama, Kanagawa 223-0033, Japan. Phone: +81-045-963-4527, Fax: +81-045-963-3977, e-mail: Chiba.Kenji@mk.mt-pharma.co.jp

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Discovery of FTY720 from a natural product, myriocin

A potent immunosuppressive natural product, myriocin was isolated from a culture broth of Isaria sinclairii, a kind of vegetative wasp that is an “eternal youth” nostrum in traditional Chinese herbal medicine⁷. Chemical modification of myriocin
yielded a new compound, 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720, f ingolimod), which has more potent immunomodulating activity and less toxicity than myriocin (Fig. 1). FTY720 at an oral dose of 0.1 mg/kg or higher significantly prolongs allograft survival in experimental skin, cardiac and renal allotransplantation models. Unlike calcineurin inhibitors, FTY720 does not impair lymphocyte function including T cell activation and production of type 1 helper T cell-associated cytokines by antigen stimulation. Moreover, we have demonstrated that FTY720 does not inhibit serine palmitoyltransferase that is the first enzyme in sphingolipid biosynthesis and is the target enzyme of myriocin. Reverse pharmacological approaches have been performed to elucidate that FTY720 is phosphorylated by sphingosine kinases (Fig. 1) and FTY720-phosphate (FTY720-P) acts as an agonist of sphingosine 1-phosphate (S1P) receptors.

Circulation of mature lymphocytes among secondary lymphoid organs (SLO), lymph and blood plays a central role in the establishment of the immune response to foreign antigens. Homing of lymphocytes from blood into SLO beyond high endothelial venules is highly dependent on the interaction between the CC-chemokine ligand (CCL) 19, CCL21, CXC-chemokine ligand (CXCL) 13, and their receptors CCR7 and CXCR5 on lymphocytes. On the other hand, throughout the analyses of the mechanism of action of FTY720, it is clarified that S1P and its receptor type 1 (S1P1) play an essential role in lymphocyte egress from SLO to lymph.

Mechanism of action of FTY720, S1P receptor modulator

FTY720 has been shown to be highly effective in various experimental allograft and autoimmune disease models including experimental autoimmune encephalomyelitis (EAE), adjuvant- or collagen-induced arthritis, and lupus nephritis. A striking feature of FTY720 is the induction of a marked decrease in the number of peripheral blood lymphocytes at doses that display an immunomodulating activity. In rats, the number of lymphocytes (T cells and B cells) in peripheral blood decreases dramatically within 6 hours after oral administration of FTY720 at 0.1 to 1 mg/kg. To clarify the mechanism of lymphocyte reduction by FTY720, distribution of lymphocytes in blood, lymph, and various SLO was analyzed after FTY720 administration in rats. When FTY720 at an oral dose of 0.1 mg/kg or higher is given to rats or mice, the number of lymphocytes is decreased markedly in the peripheral blood and thoracic duct lymph whereas that in SLO is increased significantly. Intravenous transfusion of fluorescein-labeled lymphocytes into rats reveals that the labeled lymphocytes are accumulated in SLO by FTY720 administration. These data suggest that FTY720 induces sequestration of circulating mature lymphocytes into SLO and decreases the number of lymphocytes in peripheral blood, and lymph. Thus, sequestration of circulating mature lymphocytes is presumed to be the main mechanism of immunomodulating activity of FTY720.

By reverse pharmacological approaches to clarify the mechanism of action of FTY720, it has been demonstrated that like sphingosine, FTY720 is a substrate for sphingosine kinases and
that a phosphorylated form of FTY720 (FTY720-P) acts as an agonist of S1P receptors\(^{78}\). After oral or intravenous FTY720 administration, the plasma concentration of FTY720-P was 2 to 6 times higher than FTY720 and FTY720-P is a high affinity agonist at four out of five S1P receptors\(^{78}\). S1P, a pleiotropic lysophospholipid mediator is converted primarily by the phosphorylation of sphingosine by sphingosine kinases and stimulates multiple signaling pathways resulting in calcium mobilization from intracellular stores, polymerization of actin, chemotaxis/migration, and escape from apoptosis. S1P is predominantly released by red blood cells or platelets and is found in significant amounts (100 to 400 nM) in serum. S1P binds with nanomolar affinities to five related G-protein-coupled receptors, termed S1P\(_1\)-S5.

It has been reported that S1P\(_1\) is essential for lymphocyte recirculation and that S1P\(_1\) regulates lymphocyte egress from SLO\(^{8,9,10}\). In mice whose hematopoietic cells lack a single S1P receptor, S1P\(_1\), there are no T cells in the periphery because mature T cells are unable to exit SLO\(^{9}\). Moreover, S1P\(_1\)-dependent chemotactic responsiveness is suggested to be up-regulated in lymphocytes before exit from SLO, whereas S1P\(_1\) is down-regulated during peripheral lymphocyte activation, and this is associated with retention of lymphocytes in SLO. FTY720 treatment down-regulates S1P\(_1\), creating a temporary pharmacological S1P\(_1\)-null state in lymphocytes, providing an explanation for the mechanism of FTY720-induced lymphocyte sequestration\(^{9,10}\). Since S1P\(_1\) surface expression on lymphocytes is highly dependent on the extracellular concentration of S1P, S1P\(_1\) on lymphocytes is down-regulated in the blood, up-regulated in SLO and down-regulated again in the lymph. Thus, it is proposed that cyclical modulation of S1P\(_1\) surface expression on circulating lymphocytes by S1P contributes to establishing their transit time in SLO\(^{9}\).

We have confirmed that only the (S)-enantiomer of FTY720-P can bind to four types of S1P receptors (S1P\(_1,3,4,5\)) at nanomolar concentrations, but not S1P\(_2\), whereas FTY720 up to 10000 nM does not bind S1P receptors\(^{15}\). FTY720-P shows agonist activity for S1P\(_3\) at nanomolar concentrations using extracellular signal regulated kinase 1/2 (ERK1/2) phosphorylation assay and subsequently induces long-term internalization of S1P\(_1\) in Chinese hamster ovary (CHO) cells stably expressing human S1P\(_1\) (Fig.2)\(^{6,16,17}\). The internalization of S1P\(_1\) by FTY720-P appears to be maintained longer than that by S1P and the difference between FTY720-P and S1P seems to be due to the distinct stability of FTY720-P and S1P for degradation by S1P lyase. S1P at concentrations of 10 to 100 nM induces migration of lymph node CD4 T cells in mice. By contrast, the pretreatment with FTY720-P effectively inhibits the migration of CD4 T cells toward S1P (Fig.2)\(^{6,17}\). Based on these results, it is presumed that FTY720-P converted from FTY720 markedly reduces S1P responsiveness of lymphocytes by long-term internalization of S1P\(_1\) and shows immunomodulating activity by inhibition of S1P-dependent lymphocyte egress from SLO (Fig.3).

### Pharmacological effect of FTY720 on experimental autoimmune disease models

In myelin basic protein-induced EAE in LEW rats, prophylactic administration of FTY720 at 0.1 to 1 mg/kg almost completely prevents the development of EAE symptoms, and therapeutic treatment with FTY720 significantly inhibits the progression of EAE and EAE-associated histological change in the spinal cords\(^{11}\). In myelin oligodendrocyte glycoprotein-induced EAE in DA rats, prophylactic therapy of FTY720 protects against the emergence of EAE symptoms, neuropathology, and disturbances to visual and somatosensory evoked potentials\(^{12}\). Moreover, therapeutic treatment of FTY720 markedly reverses paralysis in established EAE and normalizes the electrophysiological responses with decreased demyelination in the brain and spinal cord\(^{13}\).

Consistent with rat EAE, the development of proteolipid protein-induced EAE in SJL/J mice is almost completely prevented and infiltration of CD4 T cells into the spinal cord is decreased by prophylactic treatment with FTY720 and FTY720-P\(^{14}\). When FTY720 or FTY720-P is given after establishment of EAE in SJL/J mice, the relapse of EAE is markedly inhibited as compared with recombinant mouse interferon-\(\beta\) (rm-IFN-\(\beta\)), and the area of demyelination and the infiltration of CD4 T cells are decreased in the spinal cords of EAE mice\(^{6,15}\) (Fig.4). Similar therapeutic effects by FTY720 are obtained in the case of myelin oligodendrocyte glycoprotein-induced EAE in C57BL/6 mice\(^{16}\). These results indicate that FTY720 exhibits not only a prophylactic but also a therapeutic effect on EAE in rats and mice and that the effect of FTY720 on EAE appears to be due to a reduction of the infiltration of myelin antigen-specific CD4 T cells into the inflammation sites.

Recently it has been suggested that the efficacy of FTY720 in EAE is due to additional direct effects in the central nervous system (CNS) because neural cells constitutively express S1P receptors and relatively higher concentrations of FTY720-P are found in the CNS than blood\(^{19}\). Consequently, the therapeutic effects of FTY720 on EAE is likely due to a culmination of mechanisms involving reduction of myelin antigen-specific T cells,
neuroprotective influence of FTY720-P in the CNS, and inhibition of inflammatory mediators in the brain.

Moreover, FTY720 almost completely inhibits joint destruction and paw edema in adjuvant-induced arthritis in LEW rats and shows a marked therapeutic effect on lupus nephritis in autoimmune MRL/lpr mice. Based on these findings, S1P₁ is suggested to be a novel target for the therapy of autoimmune diseases.

Fig. 2 Effects of FTY720-P on the expression of S1P₁ and lymphocyte migration toward S1P
A: Human S1P₁-expressing CHO cells or human CD4 T cells were incubated with FTY720-P for 1 h and cell surface expression of S1P₁ was determined by flow cytometry. B: Mouse S1P₁-expressing CHO cells were incubated with FTY720-P for 3 h, and then expression of S1P₁ was observed under confocal laser microscope. C: Mouse CD4 T cells were pretreated with FTY720-P for 5 min and then migration assays toward 10 nM S1P were performed. Data are expressed as the mean ± S.E.M. of triplicate wells. Statistical differences were calculated by Dunnett’s multiple comparison test (**p<0.05, ***p<0.01). (Adapted from Maeda et al.)

Fig. 3 FTY720-P converted from FTY720 inhibits S1P/S1P₁-dependent lymphocyte egress from SLO by internalization of S1P₁ on lymphocytes

Fig. 4 Therapeutic effects of FTY720 and rm-IFN-β on EAE in SJL/J mice
SJL/J mice were immunized with myelin proteolipid protein in the presence of Freund's complete adjuvant. A: EAE-developed mice were pooled, divided into 4 groups, and administration of FTY720 (0.3 and 1 mg/kg, p.o.) or rm-IFN-β (10000 IU/mouse, intraperitoneally) was started from day 17 after immunization for 6 weeks. The results were expressed as the mean ± S.E.M. of 7 mice. Statistical differences of FTY720 groups were calculated by Steel's test (*p<0.05) and those in rm-IFN-β group were done by Mann Whitney U test (#p<0.05). B: Spinal cords of EAE-developed mice were obtained on day 28 and immunohistochemical staining of the spinal cords was performed by using anti-mouse CD4 monoclonal antibody. (Adapted from Kataoka et al.)
Therapeutic effect of FTY720 in relapsing remitting multiple sclerosis patients

It has been shown in human studies that FTY720 decreases the number of peripheral blood lymphocytes. Administration of single oral doses of FTY720, ranging from 0.25 to 3.5 mg, causes a dose-dependent reduction in peripheral blood T cells and B cells. At doses greater than 1.0 mg, the mean nadir counts are 30% to 60% below the baseline values. In multiple doses study in human, FTY720 at 1.0 mg or greater significantly reduces the number of peripheral blood lymphocytes by up to 85%, which reverses within weeks after discontinuation of the study medication.

Clinical trials of FTY720 have been performed in relapsing remitting multiple sclerosis (MS) patients. FTY720 at an oral dose of 1.25 mg or 5.0 mg, or placebo is administered daily for 6 months to 281 patients with relapsing remitting MS and total of 255 patients has completed the clinical study. The median total number of gadolinium-enhanced lesions on magnetic resonance imaging (MRI) is significantly lower with 1.25 mg and 5.0 mg of FTY720 than with placebo. The annualized relapse rates in groups given 1.25 mg and 5.0 mg of FTY720 are 0.35 and 0.36, respectively and are significantly lower than that in the placebo group (0.77). By extension study for additional 6 months, the number of gadolinium-enhanced lesions and relapse rates remains low in groups given FTY720 and both measures decrease in patients who switched from placebo to FTY720. From these results, it is demonstrated that oral FTY720 reduces the number of lesion detected on MRI and clinical disease activity in relapsing remitting MS patients. Since FTY720 possesses a novel mechanism of action and is highly effective in relapsing remitting MS patients, it is presumed that oral FTY720 provides a new therapeutic approach for autoimmune diseases including MS.

References

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