Mini Review

Therapy of autoimmune diseases by novel immunosuppressant FTY720

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FTY720 (FTY) is a new immunosuppressant which modulates sphingosine 1-phosphate receptors. FTY has been shown to be highly effective to multiple sclerosis in clinical studies. Recently, we reported the effects and the mechanisms by which FTY inhibited arthritis in rheumatoid arthritis model of SKG mice. Here, we briefly review up to date reports of FTY including our experimental results.

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Introduction

During last few years, much attention is given to sphingolipids, especially sphingosine 1-phosphate (S1P). Sphingomyelin, a major membrane sphingolipid, is hydrolyzed to ceramide which in turn is hydrolyzed to sphingosine. Sphingosine, is then phosphorylated by sphingosine kinases (SphK-1 and SphK-2) to yield S1P. S1P stimulates multiple signaling pathways resulting in calcium mobilization from intracellular stores, polymerization of actin, chemotaxis/migration, and escape from apoptosis. S1P concentrations in human and mouse plasma are 200-800 nM, where the molecule is nearly all protein-bound. Platelets had long been considered to be the major source of plasma S1P, but recent studies revealed the importance of erythrocytes as a major supply. The cellular targets of S1P were discovered approximately 10 years ago and revealed to be five G protein-coupled receptors (GPCRs), named S1P1 to S1P4 (formerly Edge1, Edge5, Edge3, Edge6, Edge8). The S1P1, S1P2, and S1P3 are expressed by a wide variety of tissues while the S1P4 and S1P5 show a more specific expression pattern. The S1P4 is mainly expressed in the hematopoietic system and lung whereas S1P5 is expressed in white matter tracts of the central nervous systems. It is well documented that S1P1 is essential for lymphocyte recirculation and that S1P1 regulates lymphocyte egress from secondary lymphoid organs (SLO) to lymph.

FTY720 (FTY) is a synthetic compound produced by modification of a metabolite from Isaria sinclairii, a kind of vegetative wasp. FTY is phosphorylated in vivo by SphK-2 to FTY-phosphate (FTY-P), which acts as a potent S1P receptors (S1PRs) agonist. These receptors are critically involved in cell survival, cytoskeletal rearrangements, cell motility, and cell migration. FTY-P binds to four types of S1PRs (S1P1, S1P3, S1P4, and S1P5) except for S1P2 and acts as a high affinity agonist at these receptors. FTY-P acts as a potent agonist at S1P1, internalizes S1P1 on lymphocytes, and inhibits the migration of lymphocytes toward S1P. This immunomodulatory effects are primarily exerted by sequestration of lymphocytes within the thymus and secondary lymphoid organs, thereby denying them the ability to recirculate to peripheral sites of inflammation. FTY-P down-modulates S1P3 in astrocytes and of S1P1 and S1P3 in oligodendrocytes, inhibiting the proliferation of these cells. FTY is also thought to act on endothelial cells by enhancing the adherence junction assembly and thus strengthening the endothelial barrier. More recent data indicate that FTY also modulates dendritic cell (DC) trafficking and function. FTY has been shown to be a useful agent for the prevention of transplant rejection and autoimmune diseases such as multiple sclerosis.

SKG mice spontaneously develop T cell–mediated chronic autoimmune arthritis as a consequence of a mutation in the gene encoding an SH2 domain of ZAP-70, a key T cell signal transduction molecule. This mutation impairs positive and negative selection of T cells in the thymus leading to thymic production of autoimmune CD4+ T cells. In addition to this disease-causing gene, polymorphisms in the major histocompatibility locus also contribute to the occurrence of SKG arthritis, depending on environmental conditions, since these mice fail to develop arthritis in sterile environments. Thus, this spontaneous autoimmune arthritis in SKG mice resembles human rheumatoid arthritis (RA) initiated by CD4+ T cells. Here we review clinical trials of FTY and experimental results of FTY including our data of SKG mice.

FTY in clinical trials

Clinical trials of FTY have been reported in renal transplantation and multiple sclerosis.

Two phase II, dose finding trials were conducted in de novo renal transplant recipients. These trials compared the efficacy of FTY and cyclosporin A (CsA) microemulsion to the standard combination therapy of mycophenolate mofetil (MMF) and CsA. FTY at 2.5mg plus full dose CsA (FDC), or 5mg FTY plus reduced dose CsA (RDC), provided equivalent efficacy as the standard therapy of MMF and FDC. Following these encouraging results, 12 month randomized double blind phase III trials were then initiated that compared the efficacy of 2.5mg FTY plus FDC or 5mg FTY plus reduced dose CsA (RDC), provided equivalent efficacy as the standard therapy of MMF and FDC. Following these encouraging results, 12 month randomized double blind phase III trials were then initiated that compared the efficacy of 2.5mg FTY plus FDC or 5mg FTY plus RDC, to MMF and FDC in de novo renal transplant patients. FTY at 2.5mg plus FDC treatment had comparable efficacy as the treatment with MMF and FDC. However, despite promising phase II results, patients receiving 5mg FTY plus RDC exhibited higher incidence of humoral acute rejection (AR). This suggested that 5mg FTY did not support a 50% reduction in CsA exposure for the prevention of AR and thus, patients in this study arm were prematurely discontinued from...
study medication\textsuperscript{25,26}.

FTY was highly effective in Phase II clinical trials with relapsing multiple sclerosis (MS)\textsuperscript{27,28}. FTY at an oral dose of 1.25mg or 5.0mg, or placebo is administered daily for 6 months to 281 patients and total of 255 patients has completed the clinical study\textsuperscript{29}. The median total number of gadolinium-enhanced lesions on magnetic resonance imaging (MRI) was lower with 1.25mg (1 lesion, P<0.001) and 5.0mg (3 lesions, P=0.006) of FTY than with placebo (5 lesions). The annualized relapse rates in groups given 1.25mg and 5.0mg of FTY are 0.35 and 0.36, respectively and are significantly lower than in the group of placebo (0.77). During the 2 year extension phase, patients who switched from placebo to FTY also showed clear reductions in annualized relapse rates and lesion counts compared with the placebo phase.

Recently, an abstract summarizing results from the 1 year Phase III TRANSFORMS study of FTY in relapsing remitting MS has been reported\textsuperscript{29}. In the study, FTY at oral dose of 0.5mg and 1.25mg showed a superior efficacy compared with a standard of care, the injectable interferon-\(\beta\). Two other ongoing studies (FREEDOMS and FREEDOMS II) are 2 years placebo-controlled Phase III studies to assess the impact of FTY in reducing the frequency of relapses and slowing the progression of disability\textsuperscript{30}. Furthermore, other Phase III study (INFORMS) of FTY is also ongoing for patients with primary progressive MS.

**Effect of FTY on autoimmune disease model**

The effect of FTY is reported in other many autoimmune disease models. Several reports have demonstrated the efficacy of FTY in the rat collagen and adjuvant-induced arthritis model. They compared the effect of FTY on arthritis with immunosuppressants such as mizoribine, cyclosporine, and tacrolimus demonstrating that the anti-arthritic effect of FTY in this model was more potent than these other immunosuppressants\textsuperscript{31,32}.

In the study using MRL-lpr/lpr (MRL/lpr) mice, which genetically predisposed to systemic lupus erythematosus (SLE), oral administration of 2 mg/kg of FTY significantly suppressed the production of anti-dsDNA antibodies and reduced the deposition of IgG in glomeruli compared to control animals. The survival rate in the FTY treated mice was 86.9% compared to 33.0% in controls\textsuperscript{33}. In the Th1-mediated 2,4,6-trinitrobenzene sulfonic acid (TNBS) colitis model mice, intraperitoneal administration of FTY reduced all clinical, macroscopic, and microscopic histopathologic parameters of colitis\textsuperscript{34}. FTY suppressed the OVA-induced Th1-mediated airway inflammation characterized by increased numbers of lymphocytes and neutrophils in bronchoalveolar lavage fluid\textsuperscript{35}. The efficacy of FTY in autoimmune diabetes\textsuperscript{36}, autoimmune myocarditis\textsuperscript{37}, and myasthenia gravis\textsuperscript{38} have also been reported.

![Fig. 1. Oral administration of FTY inhibits arthritis clinical score. Arthritis was induced by a single intraperitoneal injection of the \(\beta\)-glucan laminarin (45 mg). FTY (1 mg/kg/day) (n = 5; ●) or sterile H\(_2\)O (n = 7; ●) was administered orally at the time of laminarin injection. Changes in arthritis clinical scores were monitored over the course of 60 days. Arthritis clinical scores were monitored by inspection as follows: 0, no swelling; 0.1, swelling of one toe joint; 0.5, mild paw swelling; and 1.0, severe paw swelling. Results are expressed as mean ± SEM. *P < 0.05 vs. SKG mice given H\(_2\)O at day 60 after induction of arthritis. Representative data of two separate experiments are shown.](image)

**Effect of FTY on SKG mice**

SKG mice failed to develop RA like arthritis in strictly controlled SPF environment, although they did develop arthritis by two months under conventional microbial condition despite active thymic production of autoimmune T cells. Due to the fact that this RA model under conventional microbial conditions displays a variable onset of disease and the disease progress slowly, we induced arthritis by injection of \(\beta\)-glucan in SKG mice housed in an SPF environment. Joint swelling began to develop in a few digits approximately one month after \(\beta\)-glucan injection, subsequently progressing to other digits and to larger joints (wrists and ankles) in a symmetrical fa-
shion. In order to determine whether FTY could inhibit joint swelling, FTY (1 mg/kg) or sterile H2O as a control was administered to the mice daily by oral gavage from the time of β-glucan injection. Joint swelling of the untreated SKG mice began on day 36 after β-glucan injection and progressed significantly to day 60. In contrast, mice that received FTY showed less joint swelling as determined by the clinical score at 60 days following β-glucan injection (P < 0.05 vs. SKG mice with H2O) (Fig. 1).

Histopathology of swollen joints in the untreated SKG mice as shown by hematoxylin and eosin staining revealed vigorous proliferation of synovial cells and infiltration by mononuclear cells and neutrophils of the synovial tissues as has been observed in human RA. In contrast, these pathological changes were significantly inhibited in FTY-treated SKG mice (data not shown). X-ray examination of the ankle joints of untreated SKG mice at 60 days following β-glucan injection revealed erosion of the cartilage and subchondral bone while these changes were inhibited in FTY-treated SKG mice (data not shown).

FTY acts by selectively and reversibly sequestering lymphocytes from the peripheral blood and spleen into the lymph nodes and thymus. Therefore, we examined the effect of FTY on the numbers of peripheral blood lymphocytes, spleen cells, and thymus cells in SKG mice. Treatment with FTY resulted in a reduction of peripheral blood lymphocytes (Fig. 2A), a decrease in the spleen size (data not shown) and the numbers of spleen cells (Fig. 2A), and an increase in the thymus size (data not shown) and the
numbers of thymus cells (Fig. 2A). Significantly lower numbers of CD4+ and CD8+ T cells were found in the spleens after FTY treatment as compared with untreated mice (Fig. 2B). In contrast, we found a significant increase in the numbers of CD4+ and CD8+ T cells in the thymuses of FTY-treated mice (Fig. 2C).

Recent studies demonstrated that FTY administration prolonged the survival of solid organ allografts through retention of naive lymphocytes in secondary lymphoid organs 20,21,39). Studies utilizing the Lewis rat experimental autoimmune encephalomyelitis model demonstrated that orally administered FTY dramatically reduces clinical severity, mortality, and the infiltration of lymphocytes into the central nervous system (CNS) through retention of naive lymphocytes in secondary lymphoid organs 22. Furthermore, expression of proinflammatory cytokines such as IL-2, IL-6, and IFN-γ was markedly suppressed in the CNS following prophylactic FTY treatment 22,40). FTY rapidly induces lymphopenia through the sequestration of lymphocytes in secondary lymphoid organs and through blocking the egress of mature thymocytes from the thymus 13,14,15). Because FTY treatment results in the reduction of absolute number of peripheral blood lymphocytes, this treatment also seems to reduce the number of peripheral blood autoreactive CD4+ T cells, resulting in the reduction of autoreactive CD4+ T cell migration to autoantigen expressing joint tissues in SKG mice.

Fig. 3. Oral administration of FTY inhibits IL-6 and TNF-α expression in the synovial tissues of SKG mice. Immunohistochemistry revealed high-level expression of IL-6 and TNF-α in the synovial tissues of untreated SKG mice. In contrast, there was no detectable IL-6 or TNF-α expression in the synovial tissues of FTY-treated SKG mice. Original magnification x40. Inserts show higher magnifications of the indicated part of the synovium.

Immunohistochemical staining of synovial tissues of untreated SKG mice revealed high levels of expression of IL-6 and TNF-α. In contrast, FTY-treated SKG mice showed no expression of IL-6 or TNF-α (Fig. 3).

Effect of FTY on T cells

We examined the effect of FTY on the production of IFN-γ and IL-4 by spleen CD4+ T cells in MLR assay. Responder CD4+ T cells from Balb/c mice were cultured with irradiated (20 Gy) spleen cells from B6 mice with or without different concentrations of FTY. After 72 hours of culture, viable cells were stimulated with anti-CD3 mAb for 48 hours and the IFN-γ and IL-4 levels in the culture supernatants were assayed by ELISA. FTY slightly inhibited IFN-γ and significantly augmented IL-4 production from Balb/c CD4+ T cells stimulated by B6 spleen
cells (Fig. 4).

Data from previous studies indicated the significant immunosuppressive capacities of FTY in Th1-mediated diseases. In addition, peritoneal macrophages from FTY-treated mice displayed a decrease in classic proinflammatory M1 type macrophage activation induced by LPS and an increased anti-inflammatory M2 type macrophages activation induced by IL-4. This effect seems to be due to FTY-induced activation rather than downregulation of S1P receptors, as S1P or a specific agonist of S1P₁ (SEW2871) also blocked LPS-induced production of TNF, CCL2, IL-12, and inducible nitric-oxide synthetase. Moreover, mature DCs generated in the presence of FTY showed an impaired immunostimulatory capacity and reduced IL-12 but increased IL-10 production, indicating a shift from Th1 toward Th2 differentiation. These results represent a new aspect of the overall immunosuppressive action of FTY in which a shift from Th1 toward Th2 differentiation occurs.

**Effect of FTY on synoviocytes**

Previously, we observed that S1P levels in synovial fluid from RA patients were much higher than that from osteoarthritis patients and that S1P enhanced TNF-α- or IL-1β-induced production of PGE₂ by RA synoviocytes and MH7A cells. In the present study, we investigated the effect of FTY on the production of PGE₂ by RA synoviocytes. MH7A, an RA synoviocyte cell line, was cultured with or without different concentrations of FTY or S1P in the presence of TNF-α and PGE₂ in culture supernatant was measured by ELISA. Interestingly, S1P enhanced and FTY inhibited PGE₂ production by MH7A cells (Fig. 5). These results indicate a new mechanism for anti-inflammatory action by FTY in which FTY blocks PGE₂ production by RA synoviocytes. Studies are progress to examine the efficacy of FTY to B cell differentiation and bone metabolism in RA patients.

**Conclusion**

In this paper, we briefly reviewed up to date clinical reports about FTY including our experimental results. Many preclinical data supported that FTY has efficacy to many diseases. Recently, a notable
report that FTY suppressed ovariectomy-induced osteoporosis in mice via reducing the number of mature osteoclasts attached to the bone surface has been published\(^4\). The regulation of S1P/S1P\(_1\) signaling using FTY may become a new approach for the therapy not only of autoimmune diseases but also of many other diseases.

References


