FTY720, a new class of immunomodulator, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors

Kenji Chiba*

Abstract

FTY720 is the first of a new immunomodulator class: sphingosine 1-phosphate (S1P) receptor agonist. In 1994, an immunosuppressive natural product, ISP-I (myriocin), was isolated from the culture broth of Isaria sinclairii, a type of vegetative wasp. The chemical modification of ISP-I yielded a new compound, FTY720, which has more potent immunosuppressive activity and less toxicity than ISP-I does. FTY720 has been shown to be highly effective in experimental allotransplantation models and autoimmune disease models. A striking feature of FTY720 is the induction of a marked decrease in peripheral blood T- and B-cells at doses that show immunosuppressive activity in these models. Reportedly, FTY720 is rapidly converted to FTY720-phosphate (FTY720-P) by sphingosine kinase 2 in vivo, and FTY720-P acts as a potent agonist at S1P receptors. Recently, it has been suggested that FTY720-P internalizes S1P1 on lymphocytes and thereby inhibits the migration of lymphocytes toward S1P. Thus, it is likely that the reduction of circulating lymphocytes by FTY720 is due to the inhibition of S1P/S1P1-dependent lymphocyte egress from secondary lymphoid tissues and thymus. Because FTY720 displays a novel mechanism of action that has not been observed with other immunosuppressive agents and shows a synergism with cyclosporin A (CsA) and tacrolimus, it is presumed that FTY720 provides a useful tool for the prevention of transplant rejection and a new therapeutic approach for autoimmune diseases including multiple sclerosis and rheumatoid arthritis.

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Keywords: FTY720; Sphingosine 1-phosphate; S1P1; Immunosuppression; Lymphocyte egress

Abbreviations: AZ, azathioprine; CI, combination index; CsA, cyclosporin A; EAE, experimental autoimmune encephalomyelitis; FK506, tacrolimus; FTY720, 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride; FTY720-P, FTY720-phosphate; GvHR, graft versus host reaction; IL-2, interleukin 2; IFN-γ, interferon-γ; MHC, major histocompatibility complex antigen; MMF, mycophenolate mofetil; MST, median survival time; S1P, sphingosine 1-phosphate; S1P1, sphingosine 1-phosphate receptor type 1; (S)-FTY720-P, (S)-enantiomer of FTY720-phosphate.

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1. Introduction

It has been previously reported that a potent immunosuppressive natural product, ISP-I (myriocin), and its derivative, mycestericins, can be isolated from a culture broth of *Isaria sinclairii*, a kind of vegetative wasp that is an “eternal youth” nostrum in traditional Chinese herbal medicine (Fujita et al., 1994a, 1994b; Sasaki et al., 1994). The chemical modification of ISP-I led to a novel synthetic compound, 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720), which has more potent immunosuppressive activity and less toxicity than ISP-I (Adachi et al., 1995; Fujita et al., 1995, 1996; Hirose et al., 1996; Kiuchi et al., 2000). FTY720, at 0.1 mg/kg or higher doses, significantly prolongs skin or cardiac allograft survival and host survival in lethal graft versus host reaction (GvHR) in rats (Chiba et al., 1996; Hoshino et al., 1996). In addition, combination treatment with FTY720 and a subtherapeutic dose of cyclosporin A (CsA) or tacrolimus (FK506) results in a synergistic effect on canine renal allograft as well as rat skin or cardiac allografts (Chiba et al., 1996; Hoshino et al., 1996, 1999; Kawaguchi et al., 1996; Suzuki et al., 1996a, 1996b; Chiba & Adachi, 1997; Yanagawa et al., 1998a, 1998b). A striking feature of FTY720 is the induction of a marked decrease in the number of peripheral blood lymphocytes, especially T-cells, at doses that prolong allograft survival (Chiba et al., 1996, 1998; Hoshino et al., 1996). FTY720 does not impair lymphocyte function, including T-cell activation, but instead induces the sequestration of circulating mature lymphocytes into the secondary lymphoid tissues and decreases T-cell infiltration into grafted organs (Chiba et al., 1998, 1999; Yanagawa et al., 1998a, 1998b; Brinkmann et al., 2000). FTY720, unlike ISP-I, does not inhibit serine-palmitoyl-transferase (Fujita et al., 1996), the first enzyme in sphingolipid biosynthesis, but both molecules are structurally similar to sphingosine. Recently, it has been reported that FTY720 is effectively phosphorylated by sphingosine kinase 2 and that FTY720-phosphate (FTY720-P) is a high affinity agonist for sphingosine 1-phosphate (S1P) receptors (Brinkmann et al., 2002; Mandala et al., 2002; Paugh et al., 2003). Fig. 1 shows the chemical structures of ISP-I, FTY720, FTY720-P, sphingosine, and S1P. Moreover, it has been suggested that FTY720-P internalizes S1P receptor type 1 (S1P1) on lymphocytes and inhibits S1P/S1P1-dependent lymphocyte egress from secondary lymphoid tissues and thymus (Matloubian et al., 2004; Lo et al., 2005). This paper summarizes the current understanding of the pharmacological actions and the mechanism of action of FTY720.

2. Effect of FTY720 in experimental allograft models

FTY720 has been shown to be highly effective in prolonging allograft survival in various experimental allotransplantation models (Chiba & Adachi, 1997; Brinkmann et al., 2000). To clarify the efficacy and potency of the immunosuppressive activity of FTY720, the prolonging effect of FTY720, CsA, FK506, mycophenolate mofetil (MMF), and azathioprine (AZ) on rat skin allograft survival was compared in major histocompatibility complex antigen (MHC)-incompatible rat strains of WKAH donors and F344 recipients (Chiba et al., 1996, 1998, 2005; Fig. 2A). The immunosuppressants were administered orally for 14 days from the day of the transplantation. FTY720 at 0.03 mg/kg or higher doses significantly prolongs allograft survival in a dose-dependent manner. CsA and FK506 are also effective at doses of 3 mg/kg or more and 0.3 mg/kg or more, respectively, in this model. MMF and AZ show a marginal immunosuppressive effect only at high doses, and all
Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean survival time</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKAH to F344 control (vehicle)</td>
<td>6.6±0.2</td>
<td></td>
</tr>
<tr>
<td>FTY720, 0.1 mg/kg p.o.</td>
<td>10.5±0.3</td>
<td>&lt;0.05 vs. control</td>
</tr>
<tr>
<td>FTY720, 1 mg/kg p.o.</td>
<td>19.4±0.4</td>
<td>&lt;0.05 vs. control</td>
</tr>
<tr>
<td>CsA, 5 mg/kg p.o.</td>
<td>8.4±0.2</td>
<td>&lt;0.05 vs. control</td>
</tr>
<tr>
<td>FTY720, 0.1 mg/kg p.o. + CsA, 3 mg/kg p.o.</td>
<td>20.8±2.0</td>
<td>&lt;0.05 vs. CsA alone</td>
</tr>
<tr>
<td>FTY720, 1 mg/kg p.o. + CsA, 3 mg/kg p.o.</td>
<td>11.3±0.3</td>
<td>&lt;0.05 vs. control</td>
</tr>
<tr>
<td>FTY720, 0.1 mg/kg p.o. + FK506, 1 mg/kg</td>
<td>28.8±2.8</td>
<td>&lt;0.05 vs. FK506 alone</td>
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<td>AZ, 30 mg/kg p.o.</td>
<td>9.6±0.2</td>
<td>&lt;0.05 vs. control</td>
</tr>
<tr>
<td>AZ, 30 mg/kg p.o. + CsA, 3 mg/kg p.o.</td>
<td>10.8±0.3</td>
<td>&lt;0.05 vs. CsA alone</td>
</tr>
<tr>
<td>MMF, 100 mg/kg p.o.</td>
<td>14.6±0.6</td>
<td>&lt;0.05 vs. control</td>
</tr>
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<td>MMF, 100 mg/kg p.o. + CsA, 3 mg/kg p.o.</td>
<td>16.4±0.8</td>
<td>&lt;0.05 vs. CsA alone</td>
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<td>LEW to F344 control (vehicle)</td>
<td>8.8±0.3</td>
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</tr>
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<td>FTY720, 0.1 mg/kg p.o.</td>
<td>18.5±0.7</td>
<td>&lt;0.05 vs. control</td>
</tr>
<tr>
<td>CsA, 3 mg/kg p.o.</td>
<td>11.4±0.3</td>
<td>&lt;0.05 vs. control</td>
</tr>
<tr>
<td>FTY720, 0.1 mg/kg p.o. + CsA, 3 mg/kg p.o.</td>
<td>35.1±2.4</td>
<td>&lt;0.05 vs. CsA alone</td>
</tr>
</tbody>
</table>

Rat skin allograft was performed between WKAH or LEW donors and F344 recipients. FTY720, CsA, FK506, AZ, and MMF were administered orally for 14 days from the day of transplantation. The results were expressed as mean±SE of 8 animals, and statistical significance was calculated by generalized Wilcoxon test.

3. Synergistic effect of FTY720 in combination with calcineurin inhibitors

In clinical organ transplantations, CsA- or FK506-based combination therapy with prednisolone or other immunosuppressants is widely used to reduce the side effects of individual drugs. Therefore, it is important to investigate whether combined use of both FTY720 and CsA or FK506 produces a synergistic effect on experimental allograft models. We evaluated the concomitant effect of FTY720 with CsA or FK506 in comparison with those of AZ or MMF with CsA on acute rejection in rat skin allograft models (Chiba et al., 1996, 1998, 2005; Hoshino et al., 1999). As shown in Table 1, the combination therapy of FTY720 with CsA or FK506 has a marked prolonging effect on allograft survival as compared with the monotherapy of FTY720, CsA, or FK506. The concomitant effect of

Fig. 2. Dose-response relationship between FTY720, CsA, FK506, MMF, and AZ and skin allograft survival in MHC-incompatible and MHC-compatible rat strain systems. (A) MHC-incompatible rat skin allograft was performed using WKAH rats (RT1l) as donors and F344 rats (RT1lv1) as recipients. (B) MHC-compatible rat skin allograft was performed using LEW rats (RT1l) as donors and F344 rats (RT1lv1) as recipients. Full-thickness skin grafts (2.0 x 2.0 cm²) from donor rats were transplanted to the lateral thorax of the recipient rats. The grafts were inspected daily until rejection, which was defined as more than 90% necrosis of the graft epithelium. FTY720, CsA, FK506, MMF, and AZ were orally administered to the grafted recipients for 14 days after the transplantation.

Each symbol represents the mean±SE of the 8 animals. The statistical differences in allograft survival time compared with the vehicle-treated control group were calculated by generalized Wilcoxon test with Hommel’s multiple comparison test (*P<0.05).

Mean survival time (Day)
FTY720 with CsA or FK506 is stronger than that of AZ with CsA or MMF with CsA. To clarify the concomitant effects, the combination index (CI) values of these combination therapy groups were calculated according to the method of median effect analysis (Hoshino et al., 1999, Chiba et al., 2005). Since the CI values are less than 0.2 in the concomitant groups with FTY720 and CsA or FK506, it confirms that the combination therapy with FTY720 and CsA or FK506, exerts a synergistic effect. On the other hand, the concomitant treatment of AZ and CsA or MMF and CsA shows only an additive effect, because the CI values of these groups are 0.9 to 1.1. In MHC-compatible rat strains of LEW donors and F344 recipients, FTY720 at an oral dose of 0.1 mg/kg significantly prolongs the allograft survival and shows a synergistic effect in combination with CsA at 3 mg/kg (Table 1).

In rat heterotopic cardiac allograft model using WKAH donors and ACI recipients, the combination therapy with FTY720 and CsA or FK506 shows a more marked prolonging effect compared with that in concomitant treatment with AZ and CsA (Table 2; Hoshino et al., 1996, 1999; Chiba et al., 2005). The CI values are less than 0.2 in the concomitant group with FTY720 and CsA or FK506, indicating a synergistic effect, whereas those in the group with AZ plus CsA are 0.5 to 0.9.

Canine renal allograft survival is significantly prolonged by combination therapy of FTY720 at 0.03 and 1 mg/kg with CsA at 10 mg/kg compared with the monotherapy with FTY720 or CsA (Table 3; Kawaguchi et al., 1996; Suzuki et al., 1998; Chiba et al., 2005). With combination therapy of FTY720 and CsA, there is no severe toxicity in kidney and liver functions and the blood concentration of FTY720 or CsA (Table 3; Kawaguchi et al., 1996; Suzuki et al., 1998; Chiba et al., 2005). Since the CI values are less than 0.2 in the concomitant groups with FTY720 and CsA or FK506, it shows a synergistic effect on renal allograft survival; however, 2 dogs died during the administration period without showing increased serum creatinine and blood urea nitrogen, suggesting AZ toxicity. Moreover, it was previously reported that FTY720, in combination with a subtherapeutic dose of CsA, displays a synergistic effect on the prolongation of renal allograft survival in cynomolgus monkeys (Troncoso et al., 1999).

From these results, it is presumed that the combination therapy with FTY720 and calcineurin inhibitors provides a more beneficial tool for human organ transplantation compared with the conventional combination therapies, including calcineurin inhibitors plus AZ or MMF.

### 4. Effect of FTY720 on graft versus host disease models

When spleen cells from LEW rats are injected into the foot pad of (LEW × BN)F₁ rats, local graft versus host reaction (GVHR) is induced and the popliteal lymph node increases to its maximum weight after 7 days. FTY720 significantly inhibits the popliteal lymph node enlargement at doses of 0.1 mg/kg or more in a dose-dependent manner (Masubuchi et al., 1996). To examine the effect of FTY720 in preventing lethal GVHR, splenic lymphocytes from LEW donor rats were injected intravenously into cyclophosphamide-pretreated (LEW × BN)F₁ recipients. In the control group, all rats develop severe GVHR-associated symptoms, including redness of skin and hair loss, within 15 days after the injection of LEW spleen cells and die with a MST of 22.0 days (Masubuchi et al., 1996). The oral administration of FTY720 at 0.1 mg/kg p.o. for 30 days prevents the development of GVHR-associated symptoms and prolongs host survival significantly (MST: 50.0 days). Treatment with FTY720 at a dose of 0.3 mg/kg induces survival for more than 60 days in 4 out of 5 rats without GVHR-associated symptoms. Thus, FTY720 induces long-lasting unresponsiveness by treatment with low doses (0.1 to 0.3 mg/kg) in a
rat lethal GvHR model. Similar results are obtained in mouse lethal GvHR model using C57BL/6 donors and (C57BL/6 × DBA/2)F1 recipients (Kataoka et al., 2005).

5. Effect of FTY720 on experimental autoimmune disease models

FTY720 at 0.1 mg/kg p.o. or higher doses almost completely prevents paralysis in experimental autoimmune encephalomyelitis (EAE) induced by myelin basic protein in LEW rats (Teshima et al., 1995; Chiba & Adachi, 1997; Fujino et al., 2003). Therapeutic treatment with FTY720 inhibits EAE relapse induced by myelin proteolipid protein immunization in SJL mice (Brinkmann et al., 2002; Kataoka et al., 2004). The therapeutic potential of FTY720 is more markedly compared with recombinant mouse interferon-β (rm-IFN-β; Fig. 3A) and the area of demyelination and the infiltration of CD4+ T-cells into the spinal cord are reduced by FTY720 treatment (Kataoka et al., 2004). In the same dose range, FTY720 almost completely inhibits joint destruction as well as paw edema in adjuvant arthritis and type II collagen-induced arthritis in LEW rats (Chiba & Adachi, 1997; Matsuura et al., 2000a, 2000b). Moreover, FTY720 shows a marked prophylactic and therapeutic effect on lupus nephritis in autoimmune MRL/lpr mice (Okazaki et al., 2002; Sugahara et al., 2004). Fig. 3B shows the therapeutic effect of FTY720 on proteinuria in aged MRL/lpr mice with lupus nephritis. With only 4 weeks of FTY720 treatment at low doses, long-term improvement of lupus nephritis is observed in this autoimmune model. Moreover, therapeutic FTY720 administration decreases the number of CD4+ Th1 cells infiltrated into lupus kidney. Based on these findings, it is presumed that FTY720 can provide a new approach not only for the prevention of transplant rejection but also for the therapy of autoimmune diseases including multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus.

6. FTY720 sequesters circulating lymphocytes into secondary lymphoid tissues

A striking feature of FTY720 is the induction of a marked decrease in the number of peripheral blood lymphocytes (lymphopenia) at doses that display an...
immunosuppressive effect in experimental allograft models and autoimmune disease models. In rats, the number of lymphocytes (T-cells and B-cells) in peripheral blood decreases dramatically within 6 hr after oral administration of FTY720 at 0.1 to 1 mg/kg (Chiba et al., 1996, 1998, 1999). In particular, the reduction in T-cell numbers is remarkable. Fig. 4 shows the relationship between the number of peripheral blood lymphocytes and blood concentration after a single oral administration of FTY720 in rats. FTY720-induced lymphopenia is highly dependent on the blood concentration of FTY720. In mice, dogs, and cynomolgus monkeys, marked lymphopenia is also induced by FTY720 administration (Chiba & Adachi, 1997; Suzuki et al., 1998; Li et al., 2002; Yagi et al., 2000). Reportedly, FTY720 at 4 µM (1.4 µg/mL) or more induces apoptosis of rat spleen cells and human peripheral blood cells; however, the blood concentration range of FTY720 is lower than 100 ng/mL when given to rats at 0.1 to 1 mg/kg (Fig. 4). Thus, the hypothesis concerning FTY720-induced apoptosis is insufficient to explain the intrinsic mechanism of the decreasing effect on peripheral blood lymphocyte number by FTY720, because it is clearly impossible for FTY720 to induce apoptotic cell death of lymphocytes at a dose range of 0.1 to 1 mg/kg in vivo (Suzuki et al., 1996a, 1996b).

The immunologically mature lymphocytes are known to continuously recirculate in the blood, spleen, lymph nodes, Peyer’s patches, and lymphatic vessels. To clarify the mechanism of FTY720-induced lymphopenia, the lymphocyte distribution in blood, lymph, and various lymphoid tissues were analyzed after FTY720 administration in rats (Chiba et al., 1998; Yanagawa et al., 1998b; Chiba et al., 1999). Within 3 to 24 hr after a single oral administration of FTY720 at 0.1 to 1 mg/kg, the number of lymphocytes in rats decreased markedly in the peripheral blood, as well as in thoracic duct lymph, and partially in spleen. In contrast, at the same time, the number of lymphocytes in peripheral lymph nodes, mesenteric lymph nodes, and Peyer’s patches increased significantly (Fig. 5A). Intravenous transfusion of fluorescein-labeled rat lymphocytes into rats reveals that the labeled lymphocytes accumulate in lymph nodes and Peyer’s patches with FTY720 administration (Fig. 5B). These data suggest that FTY720-induced sequestration of circulating mature lym-

Fig. 4. The relationship between the number of peripheral blood lymphocytes and blood concentration after a single oral administration of FTY720 in rats. (A) The number of peripheral blood lymphocytes was determined by flow cytometry. Each symbol represents the mean ± SE of 6 animals. The statistical significance was calculated by Dunnett’s test as compared with the control (*P<0.05, **P<0.01). (B) The blood concentration of FTY720 was measured by gas chromatography-mass spectrometry.

Fig. 5. Effect of FTY720 on the distribution of lymphocytes in blood and lymphoid tissues in rats. (A) The number of T-cells and B-cells in the blood, thoracic duct lymph, spleen, lymph nodes, and Peyer’s patches in F344 rats was determined by flow cytometry using fluorescein-isothiocyanide (FITC)-conjugated antirat CD3 and phycoerythrin-conjugated antirat CD45RA/B monoclonal antibodies, 12 hr after a single oral administration of FTY720. Each column represents the mean of 4 animals. (B) The fluorescein-labeled lymphocytes (5×10⁷ cells) were transfused intravenously into F344 rats 2.5 hr after the oral administration of FTY720. The numbers of fluorescein-labeled lymphocytes in the blood, spleen, lymph nodes, and Peyer’s patches were determined by flow cytometry. Each column represents the mean ± SE of 4 animals. Statistical significance was calculated by Dunnett’s test (***P<0.01 vs. vehicle-treated control group).
phocytes into secondary lymphoid tissues, such as lymph nodes and Peyer’s patches, decreases the number of lymphocytes in peripheral blood, thoracic duct lymph, and spleen. Thus, the sequestration of circulating mature lymphocytes is presumed to be the main mechanism of FTY720 immunosuppressive activity.

Moreover, FTY720 reportedly inhibits mature thymocyte emigration from the thymus to the periphery in mice (Yagi et al., 2000). In the thymus, long-term daily FTY720 administration causes a 3- to 4-fold increase in the population of mature medullary thymocytes (CD4+CD8− and CD4−CD8+) as well as a slight decrease in the double-positive immature thymocyte (CD4+CD8+) ratio. An intrathymic fluorescein-labeling technique confirms that only 1/4 of the labeled cells are detected in the lymph nodes and in the spleen of FTY720-treated mice compared with the control mice. These results suggest that the immunosuppressive activity of FTY720, at least in part, could be due to its inhibitory effect on the emigration of mature thymocytes from the thymic medullar to the periphery.

7. FTY720 decreases T-cell infiltration into inflammatory sites

It is well known that calcineurin inhibitors, CsA and FK506, inhibit Th1-associated cytokines such as interleukin 2 (IL-2) and interferon-γ (IFN-γ) in alloantigen-stimulated T-cells. Unlike calcineurin inhibitors, FTY720 up to 1000 nM does not affect T-cell proliferation and Th1-associated cytokine production induced by antigen stimulation (Chiba et al., 1996, 1998; Brinkmann et al., 2000). To elucidate the mechanism of the synergistic effect of FTY720 in combination with CsA, we analyzed mRNA expression of IL-2 and IFN-γ and that of CD3, which reflects T-cell infiltration, in rat skin allograft (Yanagawa et al., 1998a). In the skin allograft, mRNA levels of IL-2, IFN-γ, and CD3 increase, peaking on days 4 to 5 after transplantation. CsA at 10 mg/kg significantly inhibits the elevation of IL-2 and IFN-γ mRNA. On the contrary, FTY720 at 0.1 mg/kg markedly inhibits the elevation of CD3 mRNA, while slightly inhibiting those of IL-2 and IFN-γ mRNA. FTY720 combined with CsA almost completely suppresses the intragraft expression of mRNA for IL-2, IFN-γ, and CD3 (Fig. 6). Immunohistochemical staining and flow cytometric analysis also confirmed that FTY720 decreases T-cell infiltration into the allograft (Yanagawa et al., 1998a, 1999, 2000). These findings suggest that unlike calcineurin inhibitors, FTY720 prolongs allograft survival by decreasing the T-cell infiltration into grafts but not Th1-associated cytokine production. In several autoimmune disease models, the reduction of T-cell infiltration into inflammatory sites is observed with FTY720 treatment (Teshima et al., 1995; Fujino et al., 2003; Kataoka et al., 2004; Sugahara et al., 2004). It is highly probable that the decreasing effect of FTY720 on T-cell infiltration into inflammatory sites is due to reduction in the number of circulating T-cells by sequestration of lymphocytes into secondary lymphoid tissues. Thus, it is presumed that the synergistic effect of FTY720 combined with calcineurin inhibitors on prolongation of allograft survival is based on the respective inhibitions of T-cell infiltration and cytokine production in allografts.

8. FTY720-phosphate converted from FTY720 acts as an agonist at S1P receptors

Two reports demonstrated that a phosphorylated form of FTY720 acts as an agonist at S1P receptors (Brinkmann et al., 2002; Mandala et al., 2002). S1P, a pleiotropic lysosphospholipid mediator, is converted primarily by phosphorylation of sphingosine by sphingosine kinase 1 and stimulates multiple signaling pathways, resulting in calcium mobilization from intracellular stores, polymer-
ization of actin, chemotaxis/migration, and escape from apoptosis (Pyne & Pyne, 2000; Hla et al., 2001). S1P is released by platelets during inflammatory processes and is found in significant amount (100 to 400 nM) in the serum (Kimura et al., 2001). S1P binds with nanomolar (nM) affinities to 5 related G-protein-coupled receptors (GPCRs), termed S1P1–5 (formerly Edg-1, -5, -3, -6, and-8). It is clear that FTY720 is a substrate for recombinant sphingosine kinase 1a and is rapidly phosphorylated in vivo (Brinkmann & Lynch, 2002; Brinkmann et al., 2002; Mandala et al., 2002). 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 4 out of 5 S1P receptors (Brinkmann et al., 2002; Mandala et al., 2002).

Recently, it has been reported that S1P1 is essential for lymphocyte recirculation and that S1P1 regulates lymphocyte egress from thymus and secondary lymphoid tissues (Brinkmann et al., 2004; Matloubian et al., 2004; Lo et al., 2005). In mice whose hematopoietic cells lack a single S1P receptor, S1P1, there are no T-cells in the periphery because mature T-cells are unable to exit the thymus and secondary lymphoid tissues (Matloubian et al., 2004). Moreover, S1P1-dependent chemotactic responsiveness is strongly up-regulated in T-cell development before exit from the thymus, whereas S1P1 is down-regulated during peripheral lymphocyte activation, and this is associated with retention in lymphoid tissues (Graler & Goetzl, 2004; Matloubian et al., 2004). FTY720 treatment down-regulates S1P1, creating a temporary pharmacological S1P1-null state in lymphocytes, providing an explanation for the mechanism of FTY720-induced lymphocyte sequestration.

After administration to rats, FTY720 is metabolized by omega-oxidation of the octyl side chain, and subsequent beta-oxidation, or phosphorylation to FTY720-phosphate (FTY720-P) by sphingosine kinase 2 rather than sphingosine kinase 1 (Paugh et al., 2003; Chiba et al., 2005). We then clarified the contribution of FTY720 metabolites including (S)-enantiomer of FTY720-P ((S)-FTY720-P) to the induction of lymphopenia and immunosuppression by FTY720 in vivo, because only (S)-FTY720-P was detected in blood from rats administered FTY720. We successfully established an effective method for asymmetric synthesis of both (S)- and (R)-enantiomers of FTY720-P with respective high enantio-selectivity and confirmed that (S)-FTY720-P binds to (S1P1, 3, 4, 5), but not S1P2, at nanomolar concentrations (Kiuchi et al., 2005; Fig. 7). On the contrary, the binding affinities of (R)-enantiomer of FTY720-P for S1P receptors are more than 100-fold weaker than those of (S)-FTY720-P. In addition, omega- and beta-oxidized 4 metabolites (M1, M2, M3, and M4) as well as FTY720 up to 10,000 nM do not bind S1P receptors. In MHC-incompatible rat skin allograft with WKAH donors and F344 recipients, (S)-FTY720-P at 0.1 and 1 mg/kg i.v. induces a marked lymphopenia and significantly prolonged the allograft survival (Chiba et al., 2005; Table 4). A similar effect of (S)-FTY720-P was observed in MHC-compatible rat skin allograft with LEW donors and F344 recipients (Table 4). On the other hand, M1, M2, M3, and M4 show

Fig. 7. Binding affinities of S1P and (S)-FTY720-P for human S1P receptors were determined by inhibition of [32P]S1P to CHO cells stably expressing S1P receptors in the presence or absence of various concentration of S1P (A) or (S)-FTY720-P (B).
Neither lymphopenia nor immunosuppression at an intravenous dose of 10 mg/kg in the rat skin allograft model. These results suggest that the lymphopenia and the immunosuppression induced by FTY720 administration are due to the agonistic activity against S1P receptors of the active metabolite, (S)-FTY720-P.

We also confirmed that (S)-FTY720-P shows agonist activity for S1P1 at nanomolar concentrations using extracellular signal regulated kinase 1/2 (ERK1/2) phosphorylation assay and subsequently induces long-term down-regulation of S1P1 in Chinese hamster ovary (CHO) cells stably expressing human S1P1. The down-regulation of S1P1 by (S)-FTY720-P appeared to be maintained longer than that by S1P (Fig. 8). Although both S1P and (S)-FTY720-P act as agonist at S1P1, it is still unclear why (S)-FTY720-P can induce long-term down-regulation of S1P1 compared with S1P. Further investigations are necessary to clarify the different actions of (S)-FTY720-P and S1P in S1P1 down-regulation. As shown in Fig. 9A and B, S1P at concentrations of 10 to 100 nM induces migration of lymph node CD4+ T-cells and CD4-single positive (SP) thymocytes toward S1P. However, (S)-FTY720-P shows agonist activity for S1P1 at nanomolar concentrations, as confirmed by the phosphorylation assay and subsequently induces long-term down-regulation of S1P1 in CHO cells stably expressing human S1P1. These results suggest that the lymphopenia and the immunosuppression induced by FTY720 administration are due to the agonistic activity against S1P receptors of the active metabolite, (S)-FTY720-P.

Table 4
Effect of (S)-FTY720-P on skin allograft survival in rats

<table>
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<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Treatment</th>
<th>Mean survival time</th>
<th>P value</th>
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<td>WKAH</td>
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<td></td>
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<td>(S)-FTY720-P, 0.1 mg/kg i.v.</td>
<td>12.1 ± 0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(S)-FTY720-P, 1 mg/kg i.v.</td>
<td>15.2 ± 0.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LEW</td>
<td>F344</td>
<td>Control (vehicle)</td>
<td>8.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(S)-FTY720-P, 0.1 mg/kg i.v.</td>
<td>24.3 ± 4.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(S)-FTY720-P, 1 mg/kg i.v.</td>
<td>31.0 ± 3.1</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Rat skin allograft was performed between WKAH or LEW donors and F344 recipients. (S)-FTY720-P was administered intravenously for 14 days from the day of transplantation. The results were expressed as mean ± SE of 8 animals, and the statistical significance was calculated as compared with vehicle-treated control group by generalized Wilcoxon test.**

Fig. 9. The migration of CD4+ T-cells and CD4-single positive (SP) thymocytes toward S1P was inhibited by (S)-FTY720-P. Lymph node CD4 T-cells or CD4 SP thymocytes (5 × 10^5 cells) were added to the upper wells of 5-μm pore polycarbonate tissue culture inserts with S1P dilution in bottom wells. Migration toward S1P was performed at 37 °C for 180 min, and migrated cells were counted by flow cytometry (A and B). (S)-FTY720-P was added to the upper well just before the migration assay toward S1P at 100 nM (C and D). Each column represents the mean ± SE of triplicate determination. Statistical significance was calculated by Dunnett’s test compared with control migration toward S1P (**P < 0.01).
S1P₁, induces the down-regulation of S1P₁ on lymphocytes, and shows immunosuppressive activity by inhibition of S1P/S1P₁-dependent lymphocyte egress from secondary lymphoid tissues and thymic medullar (Figs. 10 and 11).

10. Clinical trails of FTY720

Clinical trials of FTY20 have been performed. In phase 1a study, the administration of single oral doses of FTY720, ranging from 0.25 to 3.5 mg/kg, to stable renal transplant patients maintained on a regimen of CsA and corticosteroid, causes a dose-dependent, although transient, reduction in peripheral blood T-cells and B-cells (Budde et al., 2002). At doses greater than 1.0 mg, the mean nadir counts are 30% to 60% below the baseline values. In addition, the coadministration of single FTY720 doses does not affect CsA blood concentration. In phase 1b study on pharmacodynamics, pharmacokinetics, and safety of multiple FTY720 doses in stable renal transplant patients, FTY720 at 1.0 mg/day or greater significantly reduces peripheral blood lymphocyte count by up to 85%, which reverses within 3 days after the discontinuation of the study medication (Budde et al., 2003; Kahan et al., 2003). Compared with placebo-treated patients, FTY720 subjects show no major increase in adverse events or change in renal function. Pharmacokinetic measurements reveal that FTY720 displays linear relationship between doses and concentrations over a wide range.

The phase 2a, multicenter, open-label, dose-finding study was performed to evaluate the efficacy and safety of FTY720 compared with MMF in combination with CsA and corticosteroid in de novo renal transplant patients (Tedesco-Silva et al., 2004). The incidence of biopsy-confirmed acute rejection is 23.3%, 34.9%, 17.5%, and 9.8%, respectively, with FTY720 at doses of 0.25, 0.5, 1.0, and 2.5 mg, versus 17.1% with MMF. Safety is comparable between the FTY720 and MMF groups. Thus, FTY720 at 2.5 mg is as effective as MMF in combination with CsA for the prevention of acute rejection after renal transplantation. FTY720 is well tolerated and not associated with the side effects commonly observed with immunosuppressant therapy.

11. Conclusion

Recent published data suggest that FTY720, after phosphorylation, acts as an agonist at the S1P₁ receptor, down-regulates S1P₁ on lymphocytes, and inhibits S1P₁-dependent lymphocyte egress from secondary lymphoid tissues and thymus. Thus, FTY720 causes the sequestration of circulating mature lymphocytes into lymphoid tissues and modulates the recirculation of lymphocytes between blood and lymphoid tissues. Consequently, it is presumed that FTY720 decreases the trafficking and the infiltration of antigen-specific T-cells into grafted organs or inflammatory sites in autoimmune diseases, thereby exerting powerful immunosuppressive activity. Since FTY720 possesses a completely new mechanism of action, FTY720 may be a useful tool for the prevention of transplant rejection and a new therapeutic approach for autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus.

References


