FTY720: A Most Promising Immunosuppressant Modulating Immune Cell Functions

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Abstract: FTY720, the pharmacological analog of S1P, acts as an agonist of sphingosine-1-phosphate receptors, resulting in the inhibition of lymphocyte egress from secondary lymphoid tissues and thymocytes from the thymus, peripheral lymphopenia and interfering with normal functions of several other cell types. FTY720 has been clinically tried for transplantation and multiple sclerosis, showing promising protective effects. This review will summarize potential applications and effects of FTY720.

Key Words: FTY720, lymphocytes, monocytes, dendritic cells, endothelial cells, transplantation, autoimmune diseases, tumors.

INTRODUCTION

2-amino-2[2-(4-octylphenyl)ethy]-1,3-propanediol, known as FTY720, is a structural analog of myriocin, which is a metabolite of the ascomycete *Isaria sinclairii* that was an eternal youth nostrum in traditional Chinese herbal medicine [1]. Myriocin, also known as ISP-1, has strong immunosuppressive effects through effective inhibition of serine palmitoyl transferase, the first enzyme in the biosynthesis of sphingolipid [2]. As treatment with myriocin has shown to cause severe gastrointestinal side effects, great effort was put to the development of less toxic myriocin analogs [3]. Among the numerous synthesized myriocin derivatives, FTY720 turned out to be most promising one [3, 4]. FTY720 has more potent immunosuppressive activity and less toxicity than myriocin and it does not inhibit serine palmitoyl transferase [5]. At therapeutic relevant concentrations, FTY720 suppresses lymphocyte egress from secondary lymphoid tissues, resulting in peripheral lymphopenia but not in impairment of lymphocyte functions. FTY720 has currently been developed by Novartis Pharma AG and has shown to be highly effective in transplantation and autoimmune diseases, clinically and experimentally.

Within one hour of administration, most of FTY720 is phosphorylated by sphingosine kinases (SphK) [6] and plasma levels of phosphorylated FTY720 (FTY720-P) are 2 to 6 times higher than that of FTY720 [7, 8]. Two SphKs have been identified in mammals, SphK1 and SphK2. It has been demonstrated that FTY720 is effectively phosphorylated by SphK2 [6]. Sphingosine-1-phosphate (S1P), which is a natural lysophospholipid with high concentrations in serum [9], shares striking structural homology with FTY720-P: both having a lipophilic tail, a 2-amino group, and a phosphate head group (Fig. **1**).

S1P is mainly converted from sphingosine by SphK1 and released from the platelet during platelet activation and thrombotic processes [10], from the mast cells during inflammatory activation [11], and from other nonhematopoietic cells like endothelial cells [12]. S1P binds to five G-protein-coupled receptors (GPCRs), namely $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$ and $S1P_5$, and activates these receptors in a nanomolar range. S1P receptors were initially termed as endothelial differentiation gene receptors. $S1P_1$, $S1P_2$ and $S1P_3$ are widely expressed, with $S1P_1$ being the dominant receptor on lymphocytes. $S1P_4$ is strictly expressed by lymphoid tissues and $S1P_5$ in spleen and white matter tract of the central nervous system [13, 14]. The fundamental differences of signaling through S1P receptors rely on the variations in G-protein coupling. Two articles recently reviewed the specificity and redundancy of S1P receptors [15, 16]. Binding of S1P to its receptors activates different signaling pathways, resulting in

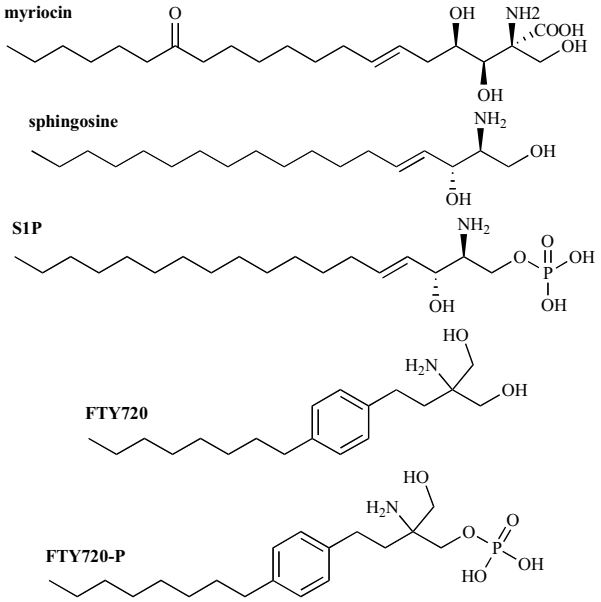

Fig. (1). Chemical structures of myriocin, sphingosine, S1P, FTY720 and FTY720-P.

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calcium mobilization from intracellular stores, polymerization of actin, chemotaxis/migration, proliferation and escape from apoptosis [13, 17]. The different activities triggered by S1P depend on the expression pattern of S1P receptors in each cell type.

FTY720-P is a potent agonist for four of the five S1P receptors, $S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$ [18]. Naïve T and B-lymphocytes express higher amounts of $S1P_1$ than $S1P_3$, $S1P_4$ and $S1P_5$. In the thymus, $S1P_1$ mRNA is highly expressed by CD4 or CD8 single positive lymphocytes but is comparatively low in CD4 and CD8 double positive cells, and is the highest in most mature, L-selectin-high single-positive thymocytes [19, 20]. $S1P_1$ is the dominant receptor on lymphocytes and the interaction of S1P with $S1P_1$ has been proven to be essential for lymphocyte (T and B cells) egress from peripheral lymphoid tissues, and for thymocyte egress from thymus [21, 22]. Naïve T cells regularly circulate between blood and lymphoid tissues. Most T and B cells need $S1P_1$ to facilitate their escape from peripheral lymphoid organs into blood and thymocytes require it to egress into circulation as well. When $S1P_1$-deficient thymocytes are transferred intravenously into wild-type recipients, these T cells conveniently enter secondary lymphoid organs but they are unable to exit. $S1P_1$-deficient B cells are however, successful in exiting the bone marrow of fetal liver chimeras and enter secondary lymphoid organs, but like T cells, they are very poor in exiting from secondary lymphoid organs. In mice, whose hematopoietic cells lack $S1P_1$, no T cells are observed in the periphery because of the inability of the mature T cells to exit the thymus and secondary lymphoid tissues [22-24]. During the course of an immune response, one day after antigen exposure, the specific lymph node T-cells had lost their responsiveness to S1P and they had down regulated $S1P_1$ expression 100-fold. Three days after immunization, many recently divided antigen-specific T-cells had appeared in the circulation, and at that time, the activated draining lymph node cells exhibited restored S1P responsiveness and increased $S1P_1$ receptor levels [14, 23]. Therefore, the egress of lymphocytes from thymus and secondary lymphoid organs depends on $S1P_1$ expression by the lymphocyte.

Exposure to FTY720-P induces $S1P_1$ down-regulation and inactivation, resulting into a functional $S1P_1$ deficient phenotype, which might be best explained by FTY720-P functioning as an $S1P_1$ agonist that cannot be readily dissociated or inactivated following receptor internalization, causing the receptor to be directed to a catabolic pathway [20, 23, 25, 26]. Therefore, it has been revealed that FTY720-P exerts immunosuppressive effects mainly through the inhibition of lymphocyte egress, resulting into peripheral lymphopenia by down-regulation of $S1P_1$ on lymphocytes [27].

This review summarizes reports on the current understanding of effects of FTY720 on different cells and discusses the potential applications of this drug in transplantation, autoimmune disorders and tumors.

EFFECTS OF FTY720 ON DIFFERENT CELL TYPES

FTY720 is phosphorylated by SphK2 to FTY720-P *in vivo*, which interacts with $S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$ to exert its immunosuppressive effects. SphK2 is essential for the immunomodulatory activity of FTY720, indicating FTY720-P to be the active metabolite following FTY720 administration [28, 29]. Due to their wide expression and complex signaling pathways of S1P receptors, FTY720 has distinguished effects on different cell types among which lymphocytes are considered to be the major target cells which have been well investigated [for reviews see 20-22, 30, 31]. However, the effects of FTY720 on non-lymphocytes are not fully investigated.

FTY720 Inhibits Lymphocyte Egress

Naïve lymphocytes continually circulate to reach secondary lymphoid tissues to survey antigens. Most naïve lymphocytes however, fail to detect antigens in one lymphoid organ and migrate to enter into the circulation after about 12-18 h for T cells and 24 h for B cells in order to survey further lymphoid organs. If the naïve lymphocytes eventually come across antigens in the secondary lymphoid organs, after activation and division, the need gradually arises to migrate from the lymphoid tissues to inflammatory sites to carry out effector functions [20]. Mature thymocytes (CD4 or CD8 single positive T cells) also need to exit the thymus to exert their functions.

Treatment with FTY720 has long been known to reduce the number of T and B cells in the blood and spleen and causes accumulation of these cells in the lymph nodes and Peyer's patches [32, 33]. In rats, one oral administration of FTY720 with therapeutic relevant concentrations (0.1 to 1 mg/kg) results in a blood concentration of FTY720 to be less than 100 ng/mL and markedly reduce lymphocyte numbers in peripheral blood within 3 to 24 h, while the numbers of lymphocytes in the secondary lymph nodes, mesenteric lymph nodes and Peyer's patches, increase significantly. Furthermore the inhibition of mature lymphocyte circulation is considered to be the major mechanism of FTY720 immunosuppressive activity [32-35]. Moreover, FTY720 inhibits egress of mature thymocytes from thymus as well. Long-term daily administration of FTY720 causes a 3- to 4-fold increase of mature thymocytes in the thymus. Using intrathymic fluorescein-labeling technique, only ¼ of fluorescein-labeled cells were found in peripheral lymph nodes and spleen, indicating that the inhibition of mature thymocyte egress from thymus also contributes to immunosuppressive activity of FTY720 [36]. Despite its egress inhibition effects on lymphocytes and thymocytes, FTY720 does not impair the activation, proliferation and effector functions of T- and B-cells at therapeutic relevant concentrations [37]. Interestingly, FTY720 was found to increase the $CD4^+/CD25^+$ regulatory T cell number *in vivo* and the suppressive activity of these regulator T cells *in vitro*, which may play a role in the immunosuppression of FTY720 [38] (Fig. **2**).

FTY720 was observed to induce apoptosis of lymphocytes at high (4 μM or more) but not at lower concentrations (lower than 100 nM). As the blood concentration of FTY720 was shown to be lower than 100 nM at therapeutic dosage (lower than 1 mg/kg daily), lymphocyte apoptosis could not be considered responsible for FTY720 effects [39, 40].

Monocytes/Macrophages

Depletion of circulating monocytes and alteration of macrophage functions following FTY720 treatment have not

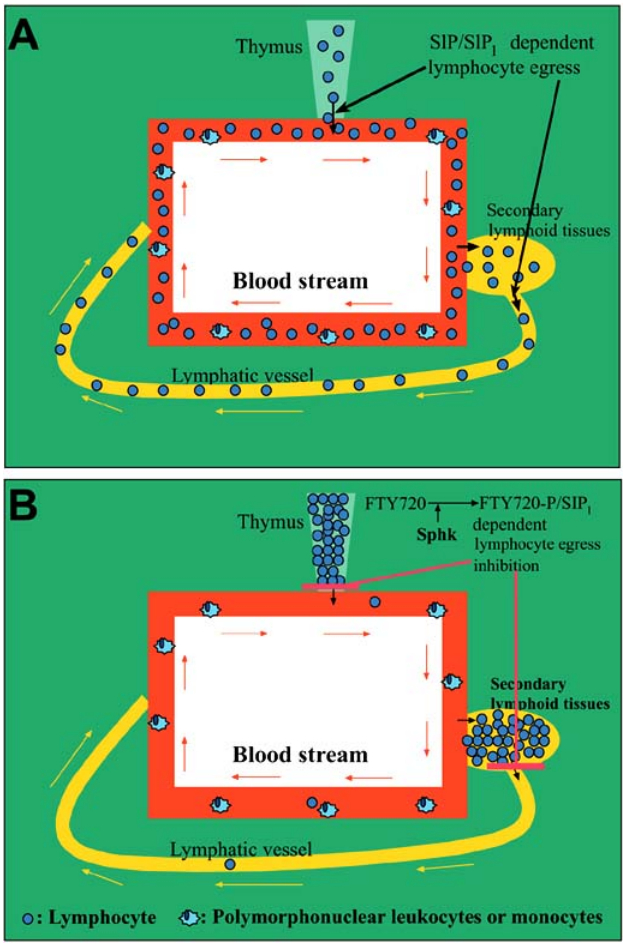

Fig. (2). Mechanisms of FTY720 inhibiting lymphocyte egress. A: Under physiological conditions, the interaction of S1P with $S1P_1$ regulates the egress of lymphocytes from secondary lymphoid nodes and thymocytes from thymus. B: FTY720 is phosphorylated by SphK to be FTY720-P after administration. FTY720-P is a potent agonist for $S1P_1$ and induces $S1P_1$ down-regulation and inactivation resulting into a functional $S1P_1$ deficient phenotype, thereby inhibiting lymphocytes egress from secondary lymphoid nodes and thymocytes egress from thymus, resulting into peripheral lymphopenia. In contrast, FTY720 does not affect the level of polymorphonuclear leukocytes or monocytes in the blood circulation.

been reported. $S1P_1$ expressed on the surface and within the cytoplasm of macrophages is not affected by FTY720-P. Overnight exposure to FTY720 significantly increases macrophage accumulation in subcapsular sinuses of FTY720-P-treated mesenteric lymph nodes [41]. Under pathological conditions, like experimental autoimmune encephalomyelitis and amyloid beta-protein stimulated monocyte infiltration, administration of FTY720 inhibits macrophage infiltration to inflammatory lesions [42, 43].

Endothelial Cells

Constitutive depletion of $S1P_1$ in mice causes embryonic lethality due to the impaired ability of vascular smooth muscle cells to migrate, cover and stabilize the nascent vascular network [44]. $S1P_3$ also plays a role in angiogenesis [45]. FTY720 is a potent agonist of $S1P_1$ and $S1P_3$ and can enhance the adherent junction assembly, strengthen the endothelial barrier and assist in preserving the integrity of the vascular system [27, 31]. *In vitro*, FTY720 causes translocation of vascular endothelial cadherin to the focal contact site between endothelial cells to promote adherent junction assembly [46]. In mice, FTY720 inhibits vascular endothelial cell growth factor causing vascular permeability [47]. *In vivo* administration of FTY720 helps in the prevention of vascular leakage and angiogenesis and has proven to be protective in abnormal-vessel-related disorders, such as endotoxin-induced inflammatory lung injury [48] and tumor angiogenesis [49, 50]. Moreover, FTY720 enhances endothelial junctional complex formation in lymph nodes, which may prevent lymphocyte leakage from lymph nodes causing peripheral lymphopenia [41].

Dendritic Cells

Dendritic cells (DCs) are the most important class of antigen-presenting cells in the immune system, holding an essential role in regulating adaptive immune responses. S1P, the endogenous analog of FTY720, mediates the migration of mature DCs [51]. Expression of all five S1P receptors was

demonstrated on murine dendritic cells (DCs). *In vivo*, administration of FTY720 greatly enhanced DC numbers in the blood, but reduces numbers in the lymph nodes and spleen. The inhibition of migratory activity of DCs by FTY720 may contribute to the immunosuppressive activity of FTY720 [52]. *In vitro*, FTY720 and FTY720-P reduce chemotaxis of DCs and thereby favor a shift from Th1 to Th2 differentiation of T cells [53]. Therefore, DCs are potent targets of FTY720.

Others Cell Types

Neutrophils

In a kidney post-transplant preservation/reperfusion injury model, FTY720 was shown to decrease neutrophil influx though the number of peripheral neutrophils remained unchanged [54]. *In vitro* evaluation showed that FTY720-P did not influence neutrophils' ability of phagocytosis and the production of reactive-oxygen species, but increased their migratory activity, suggesting that FTY720 does not inhibit critical functions of peripheral neutrophils *in vitro* [55].

Myocytes

In animal models and clinical trials, FTY720 induced a mild and transient reduction of heart rate after the initial two dosages [56, 57]. FTY720 did not reduce heart rate in $S1P_3$ deficient mice, which is expressed in rodent atrial and ventricular tissue, indicating that $S1P_3$ plays a role in the regulation of heart rate [58]. However, the high expression of $S1P_1$ but not of $S1P_3$ in human atrium and ventricle suggests a species difference [59]. Currently, FTY720 induced heart rate decrease is considered to be mediated by G protein-gated potassium channel I_{KACh} of atrial myocytes as in wild-type mice, acute FTY720 administration produced a dose-dependent, robust heart rate reduction and the heart rate reduction induced by FTY720 in I_{KACh}-deficient mice was blunted [27, 31, 60].

POTENTIAL THERAPEUTIC APPLICATIONS OF FTY720

Circulation of lymphocytes in the blood in order to enter lymph nodes, the lymph and then back to the blood is important for lymphocytes to exert immune functions. Interaction between S1P and S1P receptors is essential in regulating lymphocyte egress from secondary lymph nodes and Peyer's patches. FTY720 inhibits lymphocyte egress from secondary lymph nodes and Peyer's patches, and thymocytes from the thymus to induce significant immunosuppression in models of transplantations and autoimmune diseases. FTY720 is the first compound of a new and promising class of immunosuppressive drugs. Combination of this drug with classical immunosuppressants, like calcineurin antagonists or proliferation signal inhibitors, offers a possibility of considerable reduction in exposure to and mitigated toxicity of existing drugs. FTY720 is now progressing through human clinical trial III showing safety and tolerability in humans. Therapeutic applications of FTY720 in allograft and autoimmune diseases are the topics of several recent reviews [20-22, 30, 31, 61] and will therefore be discussed here only briefly. Application of FTY720 in tumor therapy is a recently emerging field and will be reviewed here.

Transplantation and Autoimmune Diseases

FTY720 has been shown to be effective in the inhibition of acute and chronic rejection, prolonging survival of allografted skin, kidney, heart, liver, small bowel and islet of pancreas. It has been demonstrated that FTY720 has more potent immunosuppressive activity than other immunosuppressive drugs, such as CsA, FK506, mycophenolate mofetil, and azathioprine on graft rejection in rat allograft models. Furthermore, FTY720 has synergistic effects in combination with calcineurine inhibitors. In phase Ia and IIa clinical trials, FTY720 showed promising immunosuppressive activity in renal transplantation.

Promising protective effects of FTY720 were also reported from various experimental autoimmune disease models, like myocarditis, uveoretinitis, systemic lupus erythematosus, autoimmune diabetes and experimental autoimmune encephalomyelitis (EAE). In adjuvant- or collagen-induced rat arthritis, FTY720 inhibited disease progression at doses of 0.3 mg/kg/day, showing equal or even better efficacy than mizoribine and prednisolone. The therapeutic potential of FTY720 is rather marked by the reduction of EAE, an animal model of multiple sclerosis (MS), as compared to currently used interferon-beta. Recently, a clinical trial of FTY720 in MS revealed that FTY720 reduced the number of lesions detected on magnetic resonance imaging and clinical disease activity in patients with MS [62].

Tumors

S1P is implicated in cell proliferation, transformation and migration, inhibition of apoptosis, promoting blood-vessel formation and in mediating inflammatory responses. In cancer, S1P regulates cancer-cell viability, angiogenesis and the activity of cancer-promoting factors (for example, prostaglandin E2). So, overall, S1P functions as a tumor promoter [63]. FTY720 antagonizes S1P functions and therefore has the potency to suppress tumor growth, induce tumor cell apoptosis and reduce angiogenesis.

It is already known that at a high concentration (micromolar range), FTY720 induces lymphocyte apoptosis *in vitro* and *in vivo* [40]. *In vitro*, FTY720 was shown to induce apoptosis of a variety of tumor cells, including prostate cancer DU145 [64, 65], human glioma [66], mouse breast cancer [67, 68], human T cell leukemia Jurkat [69], human bladder cancer [70], human hepatoma [71, 72] and multiple myeloma cells [73]. However, the molecular mechanisms of FYT720 induced apoptosis remain unclear and seem to promote apoptosis through modification of apoptosis regulators such as Bcl-2 and caspases [64, 65, 68, 69, 71].

In vivo, anti-cancer effects of FYT720 were tested in several tumor models, including melanoma [50], hepatocellular carcinoma [74, 49], androgen-independent prostate cancer [75], Lewis lung carcinoma [76], bladder cancer [77] and breast cancer [67] and showed significant tumor suppression. *In vivo* application of FTY720 in tumor-induced cell apoptosis inhibited tumor cell growth, suppressed angiogenesis and inhibited tumor cell migration.

CONCLUSION

FTY720 is the archetype of a new class of immunosuppressive agents that act through S1P signaling pathway to

sequestrate lymphocytes into secondary lymphatic tissues and thus away from inflammatory lesions and graft sites. FTY720 shows promising protective effects in animal models and clinical trials of transplantation, certain autoimmune diseases and tumor therapy and has proven to be well tolerated in humans. Due to its completely new mechanism of action, FTY720 is a promising member in immunosuppressive regimens.

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