A Novel Sphingosine 1-Phosphate Receptor Agonist, 2-Amino-2-propanediol Hydrochloride (KRP-203), Regulates Chronic Colitis in Interleukin-10 Gene-Deficient Mice

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ABSTRACT

Current treatments for patients with Crohn’s disease (CD) are based on recent advances in elucidating the pathophysiology of the disease. A satisfactory therapeutic strategy has not been well established. A new sphingosine 1-phosphate (S1P) receptor agonist, 2-amino-2-propanediol hydrochloride (KRP-203), has been developed for immunomodulation in autoimmune diseases and organ transplantation. We aimed to evaluate the efficacy and potency of KRP-203 on the treatment of chronic colitis in an interleukin (IL)-10 gene-deficient (IL-10-/-) mouse model. KRP-203 agonistic activity on S1P receptor was assessed in vitro. KRP-203 was administered for 1 or 4 weeks to IL-10-/- mice with clinical signs of colitis. The histological appearance of the colon and the numbers, phenotype, and cytokine production of lymphocytes were compared with a control group. KRP-203 treatment was effective in preventing body weight loss in the IL-10-/- colitis model. One-week administration resulted in the sequestration of circulating lymphocytes within the secondary lymphoid tissues. After 4 weeks of treatment, highly significant reductions were observed in number of CD4+ T cell and B220+ B cell subpopulations in the lamina propria of the colon and peripheral blood. KRP-203 obviously inhibited the production of interferon-γ, IL-12, and tumor necrosis factor-α by the colonic lymphocytes, but had no influence on IL-4 production. KRP-203 significantly inhibits ongoing IL-10-/- colitis in part through decreasing the infiltration of lymphocytes at inflammatory sites and by blocking T-helper 1 cytokine production in the colonic mucosa. Therefore, the possibility arises that KRP-203 plays a potential role in control of chronic colitis.

Crohn’s disease (CD) is generally described as a chronic inflammatory bowel disease (IBD) that occurs in genetically susceptible individuals under some specific environmental triggers, including food antigens and resident enteric bacterial flora. The precise pathogenesis remains unclear, but it may be caused by dysregulation of mucosal immune responses to enteric antigens (De Winter et al., 1999; Podolsky, 2002). Despite many therapeutic strategies so far, this disorder is considered intractable.

In interleukin (IL)-10 gene-deficient (IL-10-/-) mice, a Crohn’s-like colitis develops when the mice are bred under conventional animal care conditions, but not under germ-free conditions (Kuhn et al., 1993). Colitis in IL-10-/- mice is characterized by infiltration of activated T cells and macrophages into the lamina propria (LP) (Rennick et al., 1997). There are some evidences that CD is mediated predominantly by CD4+ TH1 cells that secrete IL-12, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α (De Winter et al., 1999; Nakase et al., 2002; Spencer et al., 2002; Elliott et al., 2004).

It was previously reported that FTY720 (FTY), a sphingosine 1-phosphate (S1P) receptor agonist, had unique properties differing from corticosteroids and calcineurin inhibitors, and it proved effective in animal models of transplantation and autoimmune disease (Brinkmann, 2004). In our previous study, FTY ameliorated the estab-
lished colitis in the IL-10−/− mouse. The mechanism responsible for this effect is an accelerated homing and sequestration of circulating lymphocytes into the Peyer’s patches (PP) and mesenteric lymph node (MLN), resulting in a reduction of peripheral blood (PB) lymphocytes and lymphocyte migration to the inflammatory site (Mizushima et al., 2004). Recently, it was reported that FTY suppresses CD4+CD44highCD62L− effector memory T cell-mediated colitis and dextran sulfate sodium-induced colitis (Deguchi et al., 2006; Fujii et al., 2006). A phase III clinical study with FTY, which was making progress in renal transplantation, has been recently suspended for side effects, such as macular edema (Salvadori et al., 2006).

We have recently discovered and synthesized a new S1P receptor agonist, KRP-203 (KRP), which has some similarities in molecular structure to FTY (Fig. 1). Furthermore, Shimizu et al. (2005) have reported that KRP prolonged graft survival and attenuated chronic rejection in rat allograft models, which suggests that KRP regulates not only T cell response but also B cell response. In our previous report, however, FTY did not affect the number of B cells (Mizushima et al., 2004). Shimizu et al. (2005) also found that a transient adverse effect, bradycardia, was less frequent with the KRP treatment. Therefore, we hypothesized that KRP may be more potent in ameliorating chronic colitis by inhibiting B cell response and T cell response, and more advantageous for long-term use. The present study was performed to evaluate the efficacy and safety of KRP on the treatment of chronic colitis in IL-10−/− mice.

**Materials and Methods**

**Calcium Mobilization Assay.** Mouse S1P1, S1P2 (GenBank accession no. NM_007901), S1P3 (NM_010101), and S1P4 (NM_010102) receptors were cloned by the polymerase chain reaction, and CHO-K1 cells stably expressing S1P receptor were subsequently established according to the method reported previously (Yu et al., 2004). Cells were plated on black/clear-bottomed 96-well assay plates at 7 × 10^4 cells/well and incubated for 24 h at 37°C in 5% CO2 atmosphere. Cells were then loaded with Calcium Kit-Fluo-3 (Dojindo, Kumamoto, Japan) for 80 min. A ligand plate was prepared by diluting S1P, phosphorylated KRP-203 (KRP-203-P; Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan), or phosphorylated FTY720 (FTY720-P; Kyorin Pharmaceutical Co., Ltd.) into 96-well plates at 10-fold the final test concentration. The ligand and cell plates were equilibrated to 37°C in the FLEX station (Molecular Devices, Sunnyvale, CA). The assay was initiated by transferring a 1/10 volume of ligand to the cell plate, and the calcium flux was recorded for 100 s. The cellular response was defined as the maximum peak height. Three independent experiments (duplicate samples per experiment) were averaged, and the results are expressed as the percentage of response activity to 1 μM of the S1P response.

**Animals.** Breeding pairs of IL-10−/− mice on a C57BL/6 background were purchased from The Jackson Laboratory (Bar Harbor, ME). Before use, the mice were maintained under specific-pathogen free conditions at the Institute of Experimental Animal Science (Osaka University Graduate School of Medicine, Osaka, Japan). Mice of both genders, aged 15 to 20 weeks, were used. Preliminary experiments demonstrated that under standard laboratory condition IL-10−/− mice developed progressive colitis from 4 weeks of age. Clinical manifestations of the disease included the passage of mucus in the stool, diarrhea, rectal prolapse, and weight loss of more than 5% of body weight.

**Drug Treatment of Mice.** KRP-203 (Kyorin Pharmaceutical Co., Ltd.) was dissolved in sterile 0.5% methyl cellulose (Wako Pure Chemicals, Osaka, Japan) solution. For in vivo treatment, the drug was administrated by gavage at a dose of 0.3 mg/kg/day to 15- to 20-week-old IL-10−/− mice that had clinical signs of colitis. Control mice received distilled water alone. At weekly intervals, the general condition and body weight of each mouse were recorded. Mice were killed by deep anesthesia with pentobarbital sodium after daily administration of KRP for 4 weeks. The numbers of lymphocytes, phenotypes, and cytokine production of KRP-treated and untreated control mice were examined.

**Isolation of Lymphocytes from Peripheral Blood, Spleen, and Mucosa-Associated Tissues.** Lymphocytes were isolated from PB, using density-separation medium (Ficoll-Paque Plus; GE Healthcare Bio-Sciences AB, Uppala, Sweden). The spleen (SP) and MLNs were aseptically removed, and single-cell suspensions were prepared using the standard mechanical disruption procedure. All MLNs were taken along the ileocecal artery, and all PP were removed from whole small intestine. Single-cell suspensions of PP and colonic lamina propria (CLP) lymphocytes were prepared by an enzymatic dissociation method, using collagenase as described previously (Fujihashi et al., 1996; Takahashi et al., 1997; Iijima et al., 1999).

**Flow Cytometric Analysis and Cell Sorting.** Immunofluorescence analysis was performed using a FACScan flow cytometer (Clontech, Mountain View, CA). Cells stained with single-color reagent were used to set the appropriate compensation levels, and at least 10,000 events were analyzed. The following monoclonal antibodies (mAbs) from BD Biosciences PharMingen (San Diego, CA) were used: anti-CD4 (RM4-5), anti-CD8α (BD Biosciences, San Diego), anti-CD44hi (RM4-5), anti-CD62L (BD Biosciences, San Diego), and anti-CD3ε (145-2C11), and anti-CD45R/B220 (RA3-6B2). For two-color flow cytometry, 1 × 10^6 cells in 20 μl of phosphate-buffered saline containing 2% fetal calf serum and 0.02% sodium azide were first incubated with anti-Fc receptor mAb (BD Biosciences PharMingen) to prevent nonspecific staining, and then they were stained with appropriate fluorescein isothiocyanate-conjugated mAb and phycoerythrin-conjugated mAb. All mAbs were used at the saturating concentrations recommended by the manufacturer. Negative control samples were stained with the irrelevant, rat isotype IgG1 antibody in parallel with the experimental samples.

Results were analyzed using Cell Quest software (BD Biosciences, San Jose, CA).

**Intracellular Cytokine Assay.** CLP and SP lymphocytes (1 × 10^6 cells/well) with 1 μg/ml soluble anti-CD28 (clone 37.51; BD Biosciences PharMingen) were cultured for 48 h in 24-well Costar plates, which had been coated with carbonate buffer, pH 9.6, containing 10 μg/ml murine anti-CD3ε mAb (clone 145-2C11; BD Biosciences PharMingen) overnight at 4°C. Supernatants were harvested and frozen at −20°C until assayed. Supernatants from culture plates were assayed for IL-4, TGF-α, and IL-12 using solid phase sandwich enzyme-linked immunosorbent assay kits (Bio-Source International, Camarillo, CA) and an IFN-γ by enzyme-linked immunosorbent assay OptEIA mouse set (BD Biosciences PharMingen). Optical densities were measured on a microplate reader (model-680; Bio-Rad, Hercules, CA) at 450 nm. Data were
analyzed by Microplate Manager, version 5.2 (Bio-Rad), and standard curves were prepared first and then the concentrations of the samples were automatically calculated.

**Histological Evaluation.** The colon was divided into three portions: proximal, middle, and distal. The tissues were fixed in 4% buffered paraformaldehyde, and then they were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

Histopathological alterations in the colonic mucosa were semi-quantified according to a modified scoring system: 1) cellular infiltration in the LP (score from 0 to 3); 2) mucin depletion (score from 0 to 2); 3) crypt abscesses (score from 0 to 2); 4) epithelial erosion (score from 0 to 2); 5) hyperemia (score from 0 to 3); and 6) thickness of the mucosa (score from 1 to 3) (Corazza et al., 1999). Hence, the range of histopathological score of each specimen was from 1 (no alteration) to 16 (most severe colitis) and that of each mouse was from 3 to 48.

**Statistical Analysis.** Results are expressed as mean ± S.E.M. Group mean values were compared by two-tailed Student’s t test. All differences were defined as significant at a value of P < 0.05.

**Results**

**KRP-203-P Has Significant Agonistic Activity on S1P1, but Almost None on S1P3.** Clinical studies with FTY showed dose-dependent asymptomatic bradycardia in stable renal transplant patients (Budde et al., 2003). This potential cardiac adverse effect is dependent on activation of S1P3 in mice (Sanna et al., 2004). To evaluate the agonistic activity of phosphorylated KRP, Ca²⁺ mobilization was measured in the CHO-K cells stably expressing mouse S1P receptors, using FLEX Station. As shown in Table 1 and Fig. 2, parent KRP and FTY had less activity, with ED₅₀ values of >1000 nM on all three S1P receptors. However, KRP-203-P and FTY720-P indicated similar agonistic activities on Ca²⁺ mobilization through S1P₁, with ED₅₀ values of 0.84 and 0.33 nM, respectively. Unlike S1P₁, KRP-203-P had no agonistic activity (ED₅₀ > 1000 nM) on S1P₃, and it had more potent activity on S1P₄ than FTY720-P.

**KRP Treatment of 4 Weeks Resulted in a Significant Reduction in the Severity of Colitis.** Control IL-10⁻/⁻ mice developed the symptoms of wasting syndrome: weight loss and hunched posture. To compare with our previous data about FTY treatment (Mizushima et al., 2004), we selected the water vehicle in control mice. However, before the experiment, we did not find any difference in terms of incidence of colitis in treated wild-type mice between 0.5% methylcellulose and water. We also used KRP in the same dose as our previous study (Mizushima et al., 2004). Mice treated with KRP in the dose of 0.3 mg/kg, however, remained in good condition and gained weight steadily (Fig. 3A). Rectal prolapse did not obviously change, but diarrhea was significantly improved from around day 14 in some treated mice. After 4 weeks, all mice treated with KRP had no diarrhea.

Macroscopically, the colonic wall in the mice treated with KRP was less thickened than that in the untreated control mice, and the sizes of the spleen and MLNs were also significantly lower (Fig. 3B). The weights of the spleen and MLNs were obviously decreased by KRP treatment, whereas colon length was significantly increased in the treated mice (Table 2).

Histologically, the intestinal lamina propria of mice treated with KRP had less elongation of gland crypts and much less infiltration of inflammatory cells than was present in the control mice. In addition, the number of goblet cells seemed almost normal in the colon of mice treated with KRP, but it was lower in the control mice (Fig. 3C).

The mean histological score of 15 to 20-week-old IL-10⁻/⁻ mice just before starting treatment was 16.1 ± 1.1 in our animal facility. After 4 weeks, the histological scores of mice treated with KRP were significantly lower than those of control mice and lower compared with start for treatment (Fig. 3D; 10.2 ± 0.85 versus 23.0 ± 0.99).

**Daily Administration of KRP for 1 Week Significantly Decreased the Number of Lymphocytes in PB, Increased It in MLN and PP, but Not in CLP.** After 1-week administration of KRP, the number of PB lymphocytes significantly decreased, compared with control mice. In contrast, the numbers of lymphocytes in the MLNs and PP were obviously higher. The number of lymphocytes in the CLP did not change (Fig. 4A).

In lymphocytes from PB, the ratios of CD3⁺/B220⁺ and CD4⁺/CD8⁺ significantly increased upon KRP treatment, whereas no difference was observed in MLN, PP, and CLP lymphocytes (Table 3).

**Daily Administration of KRP for 4 Weeks Significantly Increased the Number of Lymphocytes in PP, but Significantly Decreased It in PB and CLP.** After 4 weeks of treatment with KRP, the number of lymphocytes in PB was still significantly lower than that in control mice. The number of lymphocytes in PB was still obviously higher than that of the control animals. However, the number of lymphocytes in the MLNs returned to the control level. Most interestingly, the number of lymphocytes in CLP significantly declined compared with control values (Fig. 4B).

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### TABLE 1

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Daily Administration of KRP for 4 Weeks Significantly Decreased the Number of CD4+ T and B220+ B Cells in PB and CLP. Flow cytometric analysis performed on lymphocytes collected after 4 weeks of treatment revealed that the CD3+/B220+ and CD4+/CD8+ ratios in lymphocytes from the MLN and PP were not significantly different from the corresponding values in the control group. In contrast, the CD4+/CD8+ ratios in the lymphocytes from PB and CLP were significantly lower (Fig. 5A). Further analysis demonstrated that the numbers of CD4+ T and B220+ B cells were significantly lower in PB and CLP by KRP treatment. The number of CD8+ T cells was also significantly decreased in the PB (Fig. 5B), but no significant difference was observed in MLN and PP (data not shown).

Daily Administration of KRP for 4 Weeks Resulted in Decreased Production of IFN-γ, IL-12, and TNF-α by CLP Lymphocytes. After KRP treatment, the production of IFN-γ and TNF-α was significantly diminished in the culture media by splenocytes and CLP lymphocytes (Fig. 6, A and C) and of IL-12 production by CLP lymphocytes (Fig. 6B), but no difference was noted in IL-4 production by either lymphocyte (Fig. 6D).

Discussion

Recent advances in therapeutic strategies using mAbs against TNF-α and IL-6R or apheresis have been successful for the induction of remission in the acute phase of CD patients (Sands, 2000; Ito, 2003; Sands et al., 2006). However, to date, maintenance therapy is not satisfactory under present conditions. It is known that IL-10−/− mice spontaneously develop a Crohn’s-like enterocolitis (Kuhn et al., 1993). It has been the major focus of many studies to delineate the mechanisms underlying immune abnormalities, which play a causal role in the development of enterocolitis in IL-10−/− mice (Davidson et al., 1996; Spencer et al., 2002; Elliott et al., 2004). The enterocolitis in IL-10−/− mice is mediated by unregulated activity of CD4+ T(H)1-cells, and it is characterized by dominant T(H)1 cytokine production (Berg et al., 1996; Rennick and Fort, 2000; Nakase et al., 2002). By anti-IFN-γ
mAb or anti-IL-12 mAb treatment in IL-10−/− mice, only a slight ameliorating effect is observed on the established disease in adult mice (Davidson et al., 1998). Therefore, it seems difficult to treat established colitis only by inhibiting various cytokines. Perhaps direct regulation of activated CD4+ T cells themselves is required.

Mature lymphocytes continuously recirculate in PB, spleen, lymphatic vessels, peripheral lymph nodes, and gut-associated lymphoid tissues (Butcher and Picker, 1996). Our previous study demonstrated that FTY treatment markedly decreases the CD3+, CD4+, and CD8+ T, but not B220+ B cell subpopulations of PB and CLP lymphocytes in IL-10−/− mice compared with WT mice (Mizushima et al., 2004). Thus, not only diminishing inflammatory cytokine production in the colon but also regulating and keeping activated lymphocytes away from the effective sites may be necessary for the control of colitis in IL-10−/− mice.

FTY is structurally very similar to sphingosine, which is phosphorylated to become S1P and which mediates its effects through five G protein-coupled receptors (S1P receptors; S1P1–5) on the surface membrane of some cells. FTY is also rapidly phosphorylated in vivo by sphingosine kinase to become FTY720-P, which is biologically active. The specific role of the S1P receptor subtype has been reported, and S1P1 and S1P4 are highly expressed in T and B cells (Matloubian et al., 2004; Sanna et al., 2004). In particular, an interaction between FTY720-P and S1P1 is critically involved in lymphocyte trafficking (Pyne and Pyne, 2000). FTY720-P internalizes S1P1 on lymphocytes and abrogates S1P/S1P1-dependent egress from lymphoid organs. FTY treatment down-regulates S1P1, thereby temporarily creating a temporary pharmacological S1P1-null state in lymphocytes (Brinkmann et al., 2002). In the present study, we showed that KRP-203-P and FTY720-P have similar agonistic activities on Ca2+ mobilization through S1P1, with ED50 values of 0.84 and 0.33 nM, respectively. However, KRP-203-P had no more agonistic activity on S1P3 and a partial one on S1P4.

In the current work, we also determined that KRP treatment at a dose of 0.3 mg/kg/day for 4 weeks remarkably inhibits the development of clinical signs and histopathological changes in IL-10−/− mice with established colitis. The diarrhea was significantly improved and gained weight steadily. The histological score of KRP (10.2 ± 0.85) was almost equivalent to that of FTY (6.6 ± 0.2), as we reported previously under the same conditions (Mizushima et al., 2004).

After a 1-week treatment with KRP, the ratios of CD4+/
CD8+ and CD3+/B220+ and lymphocyte number significantly decreased in PB, but not in MLNs, PP, and LP. However, as expected, 4 week-treatment with KRP significantly decreased the number of total lymphocytes in PB and LP, and it promoted homing of these lymphocytes into the MLNs and PP. In particular, KRP remarkably reduced the number of B220+/B220+ and CD4+/CD8+ lymphocytes in the PB and LP. However, this treatment did not produce any specific effects directly on lymphocyte subpopulations in MLNs and PP (data not shown). In the previous study, FTY did not influence the number of B cells. Thus, these results indicated that KRP administration would prevent activated lymphocytes at inductive sites from migrating into LP by the sequestration mechanism. A pathogenic role for B cells was implied not only by the high frequency of these cells in inflamed tissue and elevated levels of IgG1 and IgA but also by the recent finding that sera from IL-10+/+ mice show reactivity with colon epithelial cells and LP lymphocytes (Kuhn et al., 1993; Berg et al., 1996; Davidson et al., 1996). However, enterocolitis develops in a B cell-deficient strain of IL-10−/− mice, indicating that neither B cells nor their antibody products are necessary for the initiation or perpetuation of colitis in the
IL-10⁻/⁻ mouse model (Davidson et al., 1996). There is another report in which KRP reduced the number of B220⁺ B cells and IgG deposition in the neointimal and perivascular lesions of a heart allograft (Shimizu et al., 2005). Some recent reports have indicated that the S1P receptor is highly expressed in T and B cells and that S1P₆ functions in egress of both T and B cells from peripheral lymphoid organs, including the splenic white pulp (Matloubian et al., 2004; Sanna et al., 2004). Taken together, the findings obtained from the present study suggest that KRP may prevent immunological disorders associated with both the cellular and humoral immune responses.

The TH₁ or TH₂ paradigm is important in identifying pathogenic pathways that may be operational in human and experimental IBD. There is some evidence that CD is driven by secretion of TH₁-type proinflammatory cytokines, including IL-12, IFN-γ, and TNF-α (De Winter et al., 1999; Rennick and Fort, 2000; Spencer et al., 2002; Elliott et al., 2004). IL-10⁻/⁻ mice were characterized by TH₁ cytokine profiles, and they developed transmural lesions characteristic of CD. IL-12 and IFN-γ are key mediators responsible for inducing enterocolitis in young IL-10⁻/⁻ mice, but their role in the chronic phase of disease remains uncertain. Some studies have found that anti-IL-12 mAb treatment ameliorated the ongoing disease since both the T cell number and the severity of lesions in adult IL-10⁻/⁻ mice were reduced (Davidson et al., 1998). The beneficial effect of anti-IL-12 mAb treatment was accompanied by a reduction in the number of activated CD4⁺ T cells in the intestines. In our study also, KRP significantly reduced the number of CD4⁺ T cells in the LP, and it further diminished the TH₁-type cytokines in the inflammatory sites. Compared with the untreated control IL-10⁻/⁻ mice, the production of IFN-γ and IL-12 were significantly decreased by CLP in the KRP treatment group. In contrast, no difference was observed in the production of IL-4.

Although IFN-γ was confirmed as a major mediator initiating colitis in IL-10⁻/⁻ neonates, TNF was not involved in this pathogenic process, even though abnormally high levels of TNF were detected (Davidson et al., 2000). The production of TNF-α is greater in culture CD than ulcerative colitis mucosal cells, and direct evaluation of the tissue sections by in situ hybridization shows elevated levels of TNF-α mRNA in macrophages (Cappello et al., 1992; Reinecker et al., 1993). Furthermore, it was reported that elimination of local macrophages in intestine prevents chronic colitis in IL-10⁻/⁻ mice (Watanabe et al., 2003). One potential target, nuclear factor-κB (NF-κB), was found to have high expression in macrophages from the LP of IL-10⁻/⁻ mice. NF-κB regulates the expression of various genes encoding proinflammatory cytokines. The inhibition of NF-κB activity significantly reduced weight loss and histopathological changes in IL-10⁻/⁻ mice (Neurath et al., 1996). S1P is known to activate the NF-κB signaling pathway in several cell types through Edg receptors (Siehler et al., 2001). Other investigators have reported that orally available inhibitors of sphingosine kinase can inhibit parameters of dextran sulfate sodium-induced colitis in mice (Lynn et al., 2006). KRP has been reported to inhibit macrophage infiltration into a heart allograft (Shimizu et al., 2005). In the present study, the production of TNF-α was significantly decreased by KRP treatment in LP and SP lymphocytes. KRP-203-P rapidly phosphorylated in vivo by sphingosine kinase, which internalizes S1P₁ on lymphocytes and blocks of NF-κB activity in inflammatory sites, may be associated with the attenuation of macrophage infiltration.

Tedesco-Silva et al. (2004) reported that phase 2A study was efficacious and safe in renal transplantation with FTY. However, patients who were treated with FTY were reported to develop bradycardia, impairment of pulmonary function, and macular edema. The FTY720-P binds and activates S1P receptors, resulting in lymphocyte recirculation. A recent study showed that a nonselective S1P agonist that could induce bradycardia in wild-type mice had no effect on the heart rate in S1P₆ knockout mice and that an S1P₆-selective agonist did not induce bradycardia (Sanna et al., 2004). In a rat heart transplantation model, KRP clearly showed lower tendency to cause bradycardia than FTY (Shimizu et al., 2005). This may be supported by our result that KRP activates S1P₁, but not S1P₆. Therefore, KRP has fewer adverse effects than FTY.

In summary, KRP is effective in the treatment of established colitis in adult IL-10⁻/⁻ mice. Phosphorylated KRP acts as a high-affinity agonist at S1P₁ on lymphocytes, and it accelerates sequestration of circulating lymphocytes into PP and MLNs, resulting in a reduction of CD4⁺ T cells in the inflammatory site. Moreover, KRP inhibits TH₁₆-type proinflammatory cytokine release in LP, and it ameliorates the ongoing lesions of colitis. Use of KRP with its apparent selectivity for S1P₁ and attendant action would help avoid the clinical adverse effects associated with use of FTY. Therefore, KRP might be an option to be translated into effective immunomodulation in IBD and in a variety of other models of autoimmunity disease and graft rejection.

References
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Effect of KRP-203 on Chronic Colitis in IL-10−/− Mice


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