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# Sphingosine-1-phosphate receptor agonists suppress concanavalin A-induced hepatic injury in mice

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**Abstract**

T cell-mediated immune responses play a critical role in a variety of liver injuries including autoimmune hepatitis. Injection of concanavalin A (Con A) into mice mimics the histological and pathological phenotype of T cell-mediated hepatitis. Recent advances in host immune control of organ transplantation include the development of sphingosine-1-phosphate (S1P) receptor agonists such as FTY720, which alter lymphocyte homing but do not suppress host general immunity. Herein we examined the effect of the new S1P receptor agonist KRP-203 on the Con A-induced liver damage model. In normal liver lymphocytes of BALB/c mice, both FTY720 and KRP203 promoted lymphocyte sequestering from the liver to secondary lymph nodes and significantly reduced the number of liver lymphocytes ($p < 0.05$). Based on this observation, KRP203 was employed in the Con A-induced hepatitis model. KRP203 markedly reduced the number of $CD4^+$ lymphocytes that infiltrate Con A-treated liver ($p < 0.05$) and successfully reduced serum transaminase elevation ($p = 0.017$), therefore protecting mice from Con A-induced liver injury. Interestingly this homing modulation less occurs in natural hepatic T cell homing through the chemokine receptor, CXCR4. Therefore, S1P receptor agonists preferentially target $CXCR4^+CD4^+$ peripheral blood T lymphocytes and suppress the occurrence of Con A-induced hepatitis, suggesting their therapeutic usefulness against T cell-mediated hepatic injury.


*Keywords:* Lymphocyte homing; Hepatitis; FTY720; KRP203

T cell-mediated immune responses play a critical role in a variety of liver injuries such as autoimmune hepatitis, alcohol consumption, and viral hepatitis [1–5]. Activated T cells, in fact, are detected in a variety of human liver diseases. For example, $CD4^+$ T cells represent the predominant population of T cells that infiltrate the liver in human autoimmune liver disease [6]. In the case of chronic viral hepatitis, both $CD4^+$ T and $CD8^+$ T cells have been implicated in the progression of liver injury [7–10]. Hepatic inflammation is caused by incomplete interaction between T lymphocytes and virus-harboring hepatocytes without viral elimination [10]. Similar T cell-mediated hepatitis can be induced in rodents following injection of the T cell mitogenic plant lectin concanavalin A (Con A), which rapidly induces clinical and histological evidence of hepatitis that includes elevation of transaminase activity within 8–24 h [11]. Therefore, the development of therapeutic strategies against Con A-induced hepatitis could represent a significant contribution towards the prevention or therapeutic treatment of hepatitis.

In an effort to control the host immune response in transplant patients, conventional immunosuppressants such as steroids and calcineurin inhibitors (e.g., FK506 and cyclosporine A) have been used, although use of such general immunosuppressants remains problematic in terms of infection. Recently, an S1P receptor agonist, FTY720 (2-amino-2-2-(4-octylphenyl)ethyl)

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propane-1,3-diol hydrochloride) [12,13], has been employed in organ transplantation [14,15]. Use of FTY720 offers an alternative strategy to conventional immunesuppresants employed in transplantation. FTY720 neither suppresses the whole immune status nor impairs T or B cell activation, proliferation, and memory in the host [16], and can change lymphocyte homing [14,17]. The number of peripheral lymphocytes was reduced, and these were sequestered to secondary lymphoid organs such as lymph nodes and Peyer's patches. As with FTY720, the recently developed KRP203 (2-amino-2-{3-[4-(3-benzyloxy-phenylsulfanyl)-2-chloro-phenyl]-ethyl}-propan-1,3 diol hydrochloride) acts as a similar chemical immunomodulator [18]. Its lymphocytes-sequestering activity and immunosuppressive effect were equivalent to FTY720 when employed with allograft transplantation [18].

While the effect of S1P receptor agonists on peripheral blood has been studied intensively, the effect on mononuclear cells in the liver has not been investigated to date. If S1P receptor agonists are able to reduce lymphocytes in the liver, they could be used as potential adjuncts to control hepatic injury caused by lymphocytes. Herein, we demonstrate the effect of S1P receptor agonists on mouse liver lymphocytes in Con A-induced hepatitis. In combination with our previous data [18], we propose that S1P receptor agonists may play a role in potential therapeutic strategies against T cell-mediated hepatic injury including viral hepatitis.

## Materials and methods

*Mice.* Male BALB/c mice were purchased from Charles River Japan Inc. (Kanagawa, Japan) and were used at 8–12 weeks of age. All animal experiments were performed in accordance with the Jichi Medical School guide for laboratory animals.

*Lymphocyte sequestering drugs.* KRP203 (2-amino-2-{3-[4-(3-benzyloxy-phenylsulfanyl)-2-chloro-phenyl]-ethyl}-propan-1,3-diol hydrochloride) and FTY720 (2-amino-2-2-(4-octylphenyl)ethyl) propane-1,3-diol hydrochloride) were synthesized by Kyorin Pharmaceutical Co., Ltd. (Tokyo, Japan). Both agents were dissolved in distilled water.

*Lymphocyte homing assay.* Spleen cells obtained from BALB/c mice were suspended at a final concentration of $5 \times 10^7$ cells/ml in phosphate-buffered saline (PBS). The lymphocytes were labeled with 5 μM carboxyfluorescein diacetate succinimidyl ester (CFSE) (Dojin, Kumamoto, Japan) in PBS for 5 min at room temperature [19]. After washing with PBS, cells were suspended in PBS. CFSE-labeled splenocytes ($5 \times 10^7$ cells) were injected into each BALB/c mouse (10 weeks of age) from the portal vein. Mice were sacrificed 1 h following injection.

*Con A-induced hepatitis model.* Con A was purchased from Sigma (St. Louis, MO, USA). Con A was dissolved in PBS and 20–40 mg/kg of Con A was injected intravenously in 0.2 ml PBS. Mice were sacrificed 24 h following i.v. injection of Con A. Serum transaminase activity was measured at BRL (Tokyo, Japan). Liver was perfused with 30 ml of 0.1% EDTA–PBS and liver infiltrates were recovered [20]. To remove contaminated peripheral blood lymphocytes, the first 2.5 ml of 0.1% EDTA–PBS recovered was discarded. The number of cells harvested was then counted.

*Flow cytometric analysis.* Liver mononucleocytes recovered were analyzed after lysis of red blood cells using FACS Calibur or FACS Vantage (Becton–Dickinson, San Jose, CA, USA). Data were analyzed using FlowJo (Tree Star, San Carlos, CA, USA). Anti-CD4, anti-CD8, anti-CD3, anti-CD45R/B220 (B cell marker), anti-CD11b (monocyte marker), anti-Ly-49 C (NK cell marker, clone 5E6), and anti-CD25 and anti-CXCR4 monoclonal antibodies were purchased from Pharmingen (San Diego, CA, USA). The number of each population was calculated as [total recovered cell number × proportion of lymphocytes gating × each proportion of lymphocytes gating].

*Histological examination.* Livers were fixed with 10% formalin, embedded in paraffin, sectioned, and then stained with hematoxylin and eosin. Livers were also frozen in Tissue Tek using liquid nitrogen. Cryostat sections were fixed in acetone. Sample sections were incubated overnight at 4 °C with rat anti-mouse CD4 antibody purchased from Pharmingen (San Diego, CA, USA). After washing three times with PBS, samples were incubated with biotinylated anti-rat IgG (mouse adsorbed) from Vector Laboratories (Burlingame, CA, USA) in 1/200 dilution for 1 h at room temperature. After washing three times with PBS, samples were incubated with streptavidin-Alexa488 (Molecular Probes, Eugene, OR, USA) in 1/200 dilution for 45 min and then washed with PBS.

*Real time quantitative reverse transcription (RT)-polymerase chain reaction (PCR).* Total RNA was extracted using a Cells-to-cDNAII kit (Ambion, Austin, TX, USA) from sorted $CD4^+$ T lymphocytes. SYBR green based quantitative PCR was conducted using an ABI 7700 sequence detection instrument with the following PCR primers (Takara Shuzo, Kyoto, Japan): $S1P_1$ forward, 5′-GAA CTT TGC GAG TGA GCT GGT C-3′; $S1P_1$ reverse, 5′-GGT CAG CGA GCA ATC CAA TG-3′. Results were normalized relative to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward, 5′-AAA TGG TGA AGG TCG GTG TG-3′; reverse, 5′-TGA AGG GGT CGT TGA TGG-3′.

*Statistical analysis.* Statistical analysis was performed using Fisher's PLSD test. $p$ values less than 0.05 were considered statistically significant.

## Results

### *FTY720 and KRP203 reduce the number of lymphocytes in normal liver of mice*

In an effort to examine whether FTY720 and KRP203 can reduce the number of T and B lymphocytes in normal mouse liver, liver mononuclear cells in BALB/c mice were quantified following administration of the drugs. FTY720 and KRP203 (1 mg/kg) were orally administered to mice. Liver mononuclear cells were harvested by hepatic perfusion with 30 ml of 0.1% EDTA–PBS following 24 h. Contamination by peripheral blood lymphocytes was minimized by discarding the first 2.5 ml of 0.1% EDTA–PBS recovered. The total number of recovered cells was reduced in both FTY720- and KRP203-treated mice

Table 1
Effect of FTY720 and KRP203 on normal liver infiltrates

| | Control | KRP-203 | FTY720 |
|---|---|---|---|
| Total cell | $26.4 \pm 6.2$ | $10.4 \pm 3.2^*$ | $10.7 \pm 2.5^*$ |
| $CD4^+$ | $5.8 \pm 1.0$ | $2.0 \pm 0.6^*$ | $2.0 \pm 0.6^*$ |
| $CD8^+$ | $1.9 \pm 0.6$ | $0.3 \pm 0.1^*$ | $0.5 \pm 0.3^*$ |
| $CD3^+NK^-$ | $8.0 \pm 1.4$ | $2.3 \pm 1.2^*$ | $2.8 \pm 0.8^*$ |
| $NK^+CD3^-$ | $1.6 \pm 0.5$ | $1.5 \pm 0.6$ | $0.6 \pm 0.3$ |
| $B220^+$ | $6.7 \pm 0.9$ | $1.1 \pm 0.4^*$ | $1.4 \pm 0.9^*$ |
| $CD11b^+$ | $3.1 \pm 0.6$ | $3.4 \pm 0.7$ | $3.6 \pm 0.9$ |

Peripheral blood cells from BALB/c mice ($n = 4$) were counted and analyzed by flow cytometry. The number of each proportion was calculated as [total recovered cell number × proportion of lymphocytes gating × each proportion of lymphocytes gating]. Each datum represents the mean ± standard deviation ($\times 10^5$).

* $p < 0.05$ vs. control treatment.

(Table 1). The number of $CD3^+$ T lymphocytes including $CD4^+$ and $CD8^+$ cells, and $B220^+$ B lymphocytes was also reduced in both FTY720- and KRP203-administered mice. Natural killer (NK) cells and $CD11b^+$ monocytes showed no difference irrespective of the drug used. FTY720 and KRP203 reduced the number of peripheral blood lymphocytes to 11.7% and 20.7%, respectively, compared with the mock treatment (designated as 100%). Therefore, these results demonstrate that KRP203 and FTY720 can reduce the number of T and B lymphocytes in liver as well as in the periphery.

*KRP203 promotes lymphocyte homing from liver to secondary lymph nodes*

In an effort to examine whether KRP203 could enhance lymphocyte sequestering from the liver to secondary lymph nodes, CFSE-labeled splenic lymphocytes were injected into the portal vein of mice 24 h following KRP203 administration. CFSE-labeled lymphocytes from liver, axillary and submandibular lymph nodes, peripheral blood, and spleen were analyzed by flow cytometry 1 h following injection of CFSE-labeled lymphocytes (Table 2). The proportion of $CFSE^+$ lymphocytes in $CD3^+$ T cells in the liver and peripheral blood was markedly reduced in KRP203-treated mice compared with mock-treated mice. As previously demonstrated by Shimizu et al. [18], the proportion of $CFSE^+$ lymphocytes in secondary lymph nodes was almost the same or greater in KRP203-treated mice compared with mock-treated mice. Moreover, the proportion of $CFSE^+$ lymphocytes in the spleen was far smaller in KRP203-treated mice compared with control mice. These results demonstrate that KRP203 promotes lymphocyte homing from the liver to lymph nodes.

*Preventive effect of S1P agonists on Con A-induced hepatitis*

Since FTY720 and KRP203 reduced the number of liver lymphocytes in normal mice, we set out to determine whether these S1P agonists could be effective against Con A-induced hepatitis. FTY720 or KRP203 (1 mg/kg) was orally administered to Balb/c mice 24 h prior to Con A (40 mg/kg) i.v. injection, and serum alanine aminotransferase (ALT) levels were examined 24 h following Con A injection. While FTY720-treated mice showed moderately less serum ALT levels compared with control mice ($p = 0.112$, $n = 9$) (Fig. 1), KRP203 significantly blocked an increase in ALT levels ($p < 0.05$), suggesting that KRP203 more effectively prevented Con A-induced hepatitis compared with FTY720. Thereafter, KRP203 was further investigated using the Con A-induced hepatitis model.

Microscopic examination revealed extensive infiltration of mononucleocytes and polymorphonucleocytes in the liver of Con A-treated mice, and massive necrotic areas were observed (Fig. 2A). Some hepatocytes in Con A-treated mice showed vacuolar cytoplasmic changes. In contrast, the liver of KRP203-administered mice showed far fewer mononucleocyte infiltration and necrotic areas were scarcely observed (Fig. 2B). In an effort to determine whether the liver-infiltrating mononuclear cells represent $CD4^+$ T lymphocytes, immunohistochemical analysis was performed. As shown in Fig. 2C, infiltrated mononuclear cells in Con A-treated liver of mice were positive following anti-CD4 antibody staining. A smaller number of $CD4^+$ cells were observed in KRP203-treated mice (Fig. 2D). These findings demonstrate that infiltrating mononuclear cells were mainly $CD4^+$ T cells, and that KRP203 can efficiently block $CD4^+$ T lymphocyte infiltration into the liver of Con A-treated mice.

*Dose–response of KRP203 to Con A-induced hepatic injury*

We examined the dose response of KRP203 on ALT levels in Con A-induced hepatitis (Fig. 3). Even 0.01 mg/kg of KRP203 reduced serum ALT elevation in Con A-treated mice, although the reduction was not statistically

Table 2
Lymphocyte homing in KRP203-administered mice

| | KRP-203 | Mock |
|---|---|---|
| Liver | 19.0* | 47.7 |
| PBMC | 9.5* | 86.2 |
| Spleen | 7.6* | 4.6 |
| Axillar LN | 2.3* | 1.7 |

KRP-203 or PBS was administered orally and CFSE-labeled splenocytes ($5 \times 10^7$ cells) were injected from the portal vein of mice at 24 h later. $CFSE^+$ lymphocyte proportion (%) in $CD3^+$ T lymphocytes 1 h after the portal injection was measured by a flow cytometer.

* $p < 0.05$ vs. mock treatment.

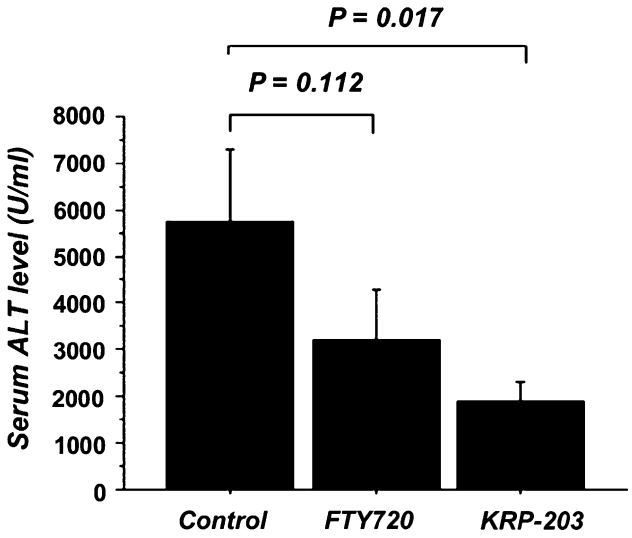


Fig. 1. Effect of FTY720 and KRP-203 on Con A-induced hepatitis. Con A (40 mg/kg) was injected intravenously into the tail vein of mice 24 h following oral administration of either FTY720 or KRP203 (1 mg/kg). Mice were sacrificed 24 h following Con A injection and serum ALT activity was measured. The data represent means ± standard deviation ($n = 9$–10 per group).

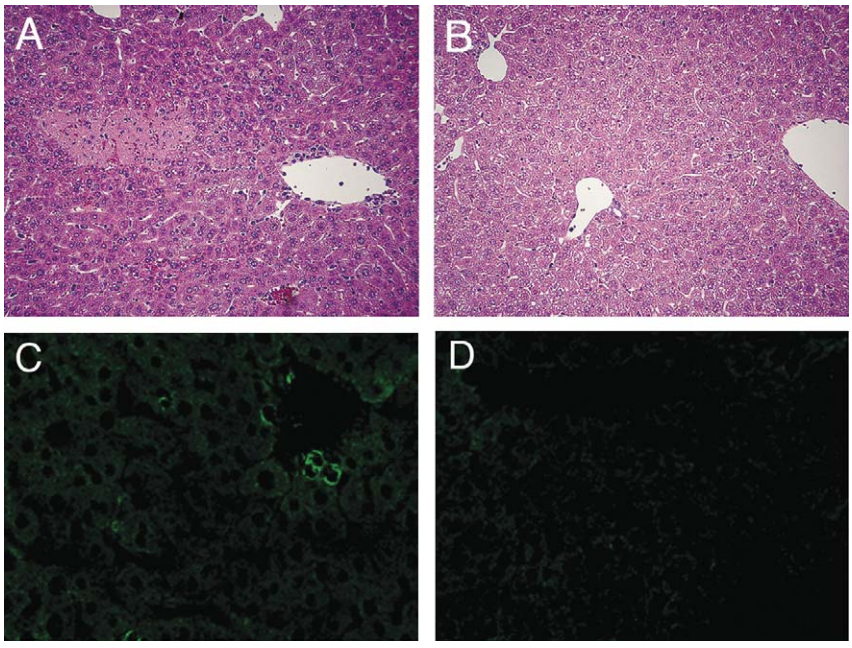


Fig. 2. Histology of Con A-induced hepatitis with KRP-203 administration. Light micrographs of liver in Con A-treated mice (A) and KRP203-administered Con A-treated mouse (B) (hematoxylin and eosin staining, original magnification 100×). Immunostaining of liver with anti-CD4 antibody in Con A-treated mice (C,D). Frozen sections of liver 24 h following Con A injection were stained with rat anti-mouse CD4 antibody followed by treatment with biotinylated anti-rat IgG (mouse adsorbed) and streptavidin-Alexa488. (C) Control Con A-treated mouse; (D) KRP-203-administered Con A-treated mouse. Original magnification 200×.

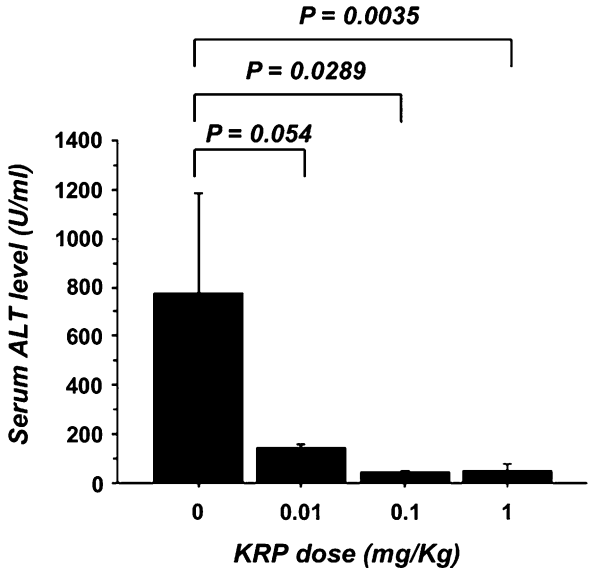


Fig. 3. Population of lymphocytes in the Con A-treated BALB/c mice liver. Mononuclear cells recovered from Con A-treated BALB/c mice ($n = 4$) were analyzed using flow cytometry. The data demonstrate the representative plots (1 of 3 independent experiments with similar results). The percentages of each population of lymphocytes gating are shown in each quadrant.

significant ($p = 0.054$). However, higher doses of KRP203 (0.1 and 1.0 mg/kg) almost completely inhibited serum ALT elevation, compared with the control treatment ($p = 0.0289$ and 0.0035, respectively). Therefore, these results indicate that KRP203 can protect mice from Con A-induced hepatic injury at a relatively higher dose.

### *Flow cytometric analysis of hepatic mononuclear cells in Con A-induced liver injury*

In an effort to evaluate the effect of KRP203 on liver mononucleocytes in Con A-induced hepatitis, hepatic mononuclear cells 24 h following Con A injection were isolated and analyzed by flow cytometry (Fig. 4). The total number of cells harvested was significantly reduced in KRP203-treated mice (1 mg/kg) (Table 3), and the proportion of $CD3^+$ and $CD4^+$ T lymphocytes was also reduced in KRP203-treated animals. However, the reduction in the number of $CD8^+$ cells, which represents both $CD8^+$ T and NK/T cells, was not as large as that observed for $CD3^+$ and $CD4^+$ lymphocytes. These quantitative studies demonstrate that KRP203 can preferentially reduce the $CD4^+$ T lymphocyte population in Con A-induced hepatic injury.

### *Effect of KRP203 on liver $CD4^+$ T lymphocytes*

In an effort to determine whether KRP203 could affect S1P receptor 1 expression in the same number of liver $CD4^+$ T lymphocytes, a real-time quantitative RT-PCR study was performed. S1P receptor 1 mRNA was expressed at the steady-state level in hepatic $CD4^+$ T lymphocytes, but the expression remained unchanged following KRP203 administration (Fig. 5A). Furthermore, since expression of the homeostatic chemokine receptor CXCR4 can control natural homing of liver $CD4^+$ lymphocytes, their proportion was examined by a flow cytometry

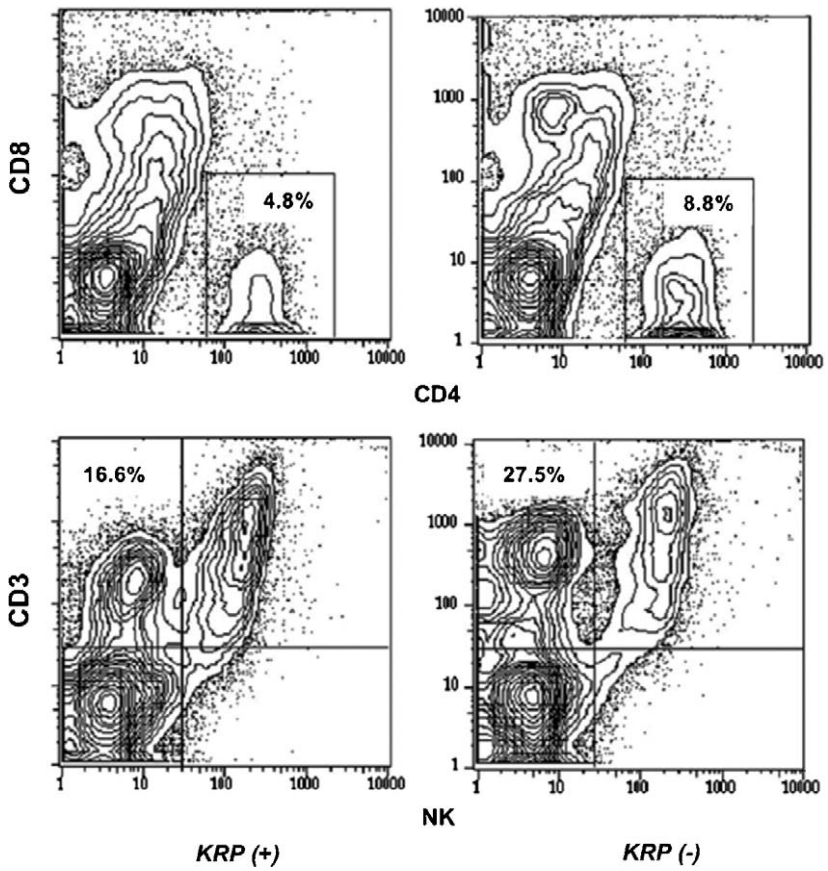


Fig. 4. Dose–response of KRP-203 on ALT activity in Con A-induced hepatitis. Con A was injected intravenously 24 h following oral administration of the indicated dose of KRP-203. Mice were sacrificed 24 h following Con A injection and serum ALT activity was evaluated. The data represent the mean ± standard error ($n = 4$; 1 of 3 independent experiments with similar results).

Table 3
Effect of KRP-203 on liver infiltrates in Con A-treated mice

| | Control | KRP-203 |
|---|---|---|
| Total cell | 31.8 ± 8.4 | 16.3 ± 3.5* |
| $CD4^+$ | 3.0 ± 1.0 | 0.9 ± 0.3* |
| $CD8^+$ | 3.9 ± 1.0 | 2.5 ± 0.6* |
| $CD3^+NK^-$ | 8.2 ± 2.3 | 2.3 ± 0.5* |
| $NK^+CD3^-$ | 0.5 ± 0.2 | 0.4 ± 0.2 |
| $B220^+$ | 6.2 ± 2.1 | 2.1 ± 0.2* |
| $CD11b^+$ | 10.7 ± 4.4 | 8.5 ± 2.8 |

Blood cells recovered from Con A-treated BALB/c mice were counted and analyzed using a flow cytometer. The number of each proportion was calculated as [total recovered cell number × proportion of lymphocytes gating × each proportion of lymphocytes gating]. Each datum represents the mean ± standard deviation ($\times 10^5$).

* $p < 0.05$ vs. control treatment.

(Fig. 5B). Peripheral blood lymphocytes of KRP203-treated mice contained less $CD4^+$ T cells (especially $CXCR4^+CD4^+$ T cells) than control mice, suggesting that the $S1P_1$ agonist targeted $CD4^+$, especially $CXCR4^+CD4^+$ peripheral T cells. However, $CXCR4^+CD4^+$ T cells in the liver were not affected by KRP203. In fact, the CXCR4 ligand, stromal cell-derived factor-1 (SDF-1/CXCL12), was expressed much in the liver (data not shown) [21]. This suggests that KRP203 does not affect the natural recruitment of $CXCR4^+CD4^+$ T cells in the microenvironment of the liver.

## Discussion

The present investigation demonstrated that use of the S1P receptor agonists FTY720 and KRP203 blocked the occurrence of Con A-induced hepatic injury in BALB/c mice. These S1P receptor agonists reduced the number of T lymphocytes in peripheral blood and in the liver, and sequestered T lymphocytes to the lymph nodes of mice. Application of this lymphocyte sequestering potential to Con A-induced hepatitis demonstrated a reduction in ALT elevation and attenuated microscopic liver damage. Flow cytometric analysis revealed that KRP203 could preferentially reduce $CXCR4^+CD4^+$ T lymphocyte infiltration into damaged liver. These results suggest that S1P receptor agonists suppress the occurrence of Con A-induced hepatitis by targeting $CXCR4^+CD4^+$ T lymphocytes.

S1P receptor agonists reduce the number of T lymphocytes in peripheral blood by the lymphocyte sequestering effect to secondary lymph nodes [12,14,17,18]. This potential mechanism of S1P receptor agonists suggests that circulating T lymphocytes reduce infiltration of alloreactive T lymphocytes into grafts and subsequently prolong graft

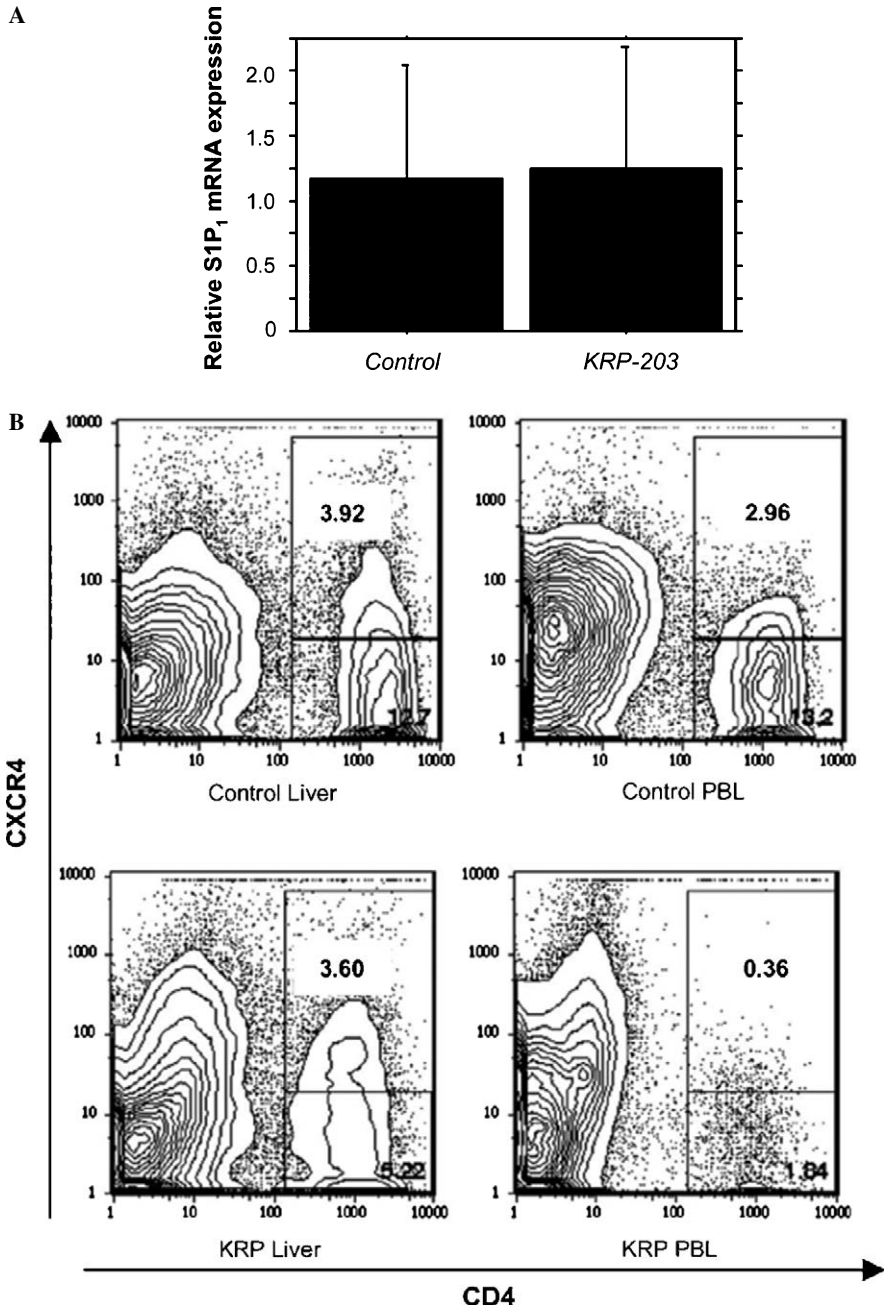


Fig. 5. Expression of S1P receptor 1 and CXCR4 in Con A-treated $CD4^+$ lymphocytes. (A) Quantitative RT-PCR analysis of sphingosine-1-phosphate receptor 1 in sorted liver $CD4^+$ T lymphocytes recovered from Con A-treated and control BALB/c mice (each $n = 4$). Data were normalized to GAPDH. The data represent means ± standard error. (B) Flow cytometric analysis of liver and peripheral blood lymphocytes recovered from Con A-treated and control BALB/c mice. Data are the representative plots with similar results. The percentages of each population of lymphocytes gating are shown in each quadrant.

survival [12,22]. Indeed, FTY720 is very effective when employed in organ transplantation throughout the duration of clinical studies [23] and has the advantage of not inducing nonspecific immunosuppression such as that observed with the use of calcineurin inhibitors [24]. FTY720 does not impair T and B cell activation, expansion, and memory, but only reduces $CD8^+$ cytotoxic T lymphocyte recirculation [16]. This effect of immuno-modulation suggests a broad application of these drugs in cases concerning various inflammatory diseases, and is not to limited to organ transplantation.

Although the increase in $CFSE^+$ lymphocytes in the peripheral lymph nodes was modest (Table 2), it was due to saturated lymphocytes sequestering by KRP203 pretreatment before $CFSE^+$ lymphocyte administration. The tendency of lymphocyte sequestering was reproducible, and the lymphocyte sequestering effect of KRP203 has been enough established [18]. KRP203 elicits lymphopenia

and is very effective in skin and heart transplantation. To date, the effectiveness of FTY720 has only been reported in various experimental autoimmune disease models such as arthritis [25], myocarditis [26], systemic lupus erythematosus [27], and thyroiditis [28]. Nonetheless, its effect on normal healthy organs had not been demonstrated, nor the effect of FTY720 and KRP203 on liver. The liver represents one of the hematopoietic organs until neonatal age and contains many mononuclear cells. Interestingly, our quantitative study showed that both KRP203 and FTY720 reduced the number of lymphocytes even in the normal liver of mice. KRP203 administration promoted lymphocyte homing from the liver to lymph nodes, as well as from peripheral blood, and resulted in a reduced number of liver lymphocytes, as in the periphery. This evidence also suggests that the liver does not act as a lymphocyte-sequestrating secondary lymphoid organ.

Among the various hepatitis models, it is known that Con A-induced hepatitis is mediated by T lymphocyte activation [11]. Pretreatment with anti-CD4 antibody inhibited Con A-induced hepatic injury, and SCID mice and athymic nude mice were also protected from Con A-induced liver damage. Administration of general immunosuppressants such as dexamethasone and calcineurin inhibitors can protect mice from severe Con A-induced hepatitis [11]. However, use of these general immunosuppressants for benign diseases such as chronic viral hepatitis is cautious for fear of infection. FTY720 and KRP203 belong to a group possessing a different pharmacological mode of immunosuppressant action, and spatial sequestering of T lymphocytes by these S1P receptor agonists appears to safely relieve immune cell-mediated hepatitis.

KRP203 reduced the $CXCR4^{+}CD4^{+}$ T lymphocyte population in peripheral blood. The homeostatic chemokine CXCL12/SDF-1 is highly expressed in the liver [21] and the proportion of $CXCR4^{+}CD4^{+}$ T lymphocytes in the liver was slightly higher than that originally in peripheral blood (Fig. 5B). KRP203 did not affect the expression of CXCR4 in peripheral blood $CD4^{+}$ T lymphocytes (data not shown), and a similar tendency was observed with FTY720 treatment [29]. Furthermore, the relative proportion of $CXCR4^{+}CD4^{+}$ T lymphocytes versus $CXCR4^{-}CD4^{+}$ T lymphocytes increased in the liver following KRP203 administration. Although KRP203 targets peripheral $CXCR4^{+}CD4^{+}$ T lymphocytes, it is possible that relative increase in number of hepatic $CXCR4^{+}CD4^{+}$ T lymphocytes might result from the abundant expression of CXCL12/SDF-1 in the liver.

In conclusion, the modulation of hepatic T lymphocytes by S1P receptor agonists attenuates the occurrence of Con A-induced hepatitis by reducing $CXCR4^{+}CD4^{+}$ T lymphocyte infiltration. The effectiveness of these lymphocyte-sequestering drugs on T cell-mediated liver disease models suggests their potential usefulness. This new pharmacological action provides a promising new therapeutic option against T lymphocyte-mediated liver injury.

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