KRP-203, a Novel Synthetic Immunosuppressant, Prolongs Graft Survival and Attenuates Chronic Rejection in Rat Skin and Heart Allografts

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Background—A novel immunomodulator, KRP-203, the molecular structure of which has some similarity to FTY720, has been developed for use in organ transplantation. The present study was designed to investigate the potency and safety of KRP-203 on allograft survival against both acute and chronic rejection in rat skin and heart transplantation.

Methods and Results—KRP-203 significantly prolonged skin or heart allograft survival of a minor histocompatibility complex (mHC)–disparate (LEW to F344) rat combination. Histopathological and immunohistochemical analysis at 100 days after mHC-disparate rat heart transplantation revealed that KRP-203 treatment significantly inhibited infiltration of inflammatory cells, including macrophages and T cells; expression of endothelin-1 and transforming growth factor-β1; and IgG deposition and eventually attenuated neointimal formation and myocardial fibrosis. KRP-203 also prolonged heart allograft survival in a major histocompatibility complex (MHC)-incompatible (DA to LEW) rat combination, but the efficacy was not as significant. However, KRP-203 combined with a subtherapeutic dose of cyclosporin A synergistically prolonged the heart allograft survival. Flow cytometric analysis demonstrated that KRP-203 reduced the number of peripheral blood mononuclear cells (lymphocytes and monocytes) but not granulocytes and enhanced lymphocyte homing into peripheral lymph nodes. The influence of KRP-203 on heart rate changes in Hartley guinea pigs was examined. KRP-203 had less of a tendency to cause bradycardia than FTY720.

Conclusions—These findings demonstrated that KRP-203 prolonged skin and heart allograft survival and significantly attenuated chronic rejection and bradycardia as an adverse effect. Therefore, KRP-203 offers considerable potential as a novel therapeutic immunosuppressant in patients with organ transplantation. (Circulation. 2005;111:222-229.)

Key Words: arteriosclerosis ■ rejection ■ transplantation ■ heart rate

The development of immunosuppressants has enabled patients with organ dysfunction to receive organ transplantation. Calcineurin inhibitors such as cyclosporin A (CsA) and tacrolimus (FK506) were introduced to clinical use in the 1980s and have improved graft and patient survival after organ transplantation. Despite the use of these drugs, clinical transplantation has not achieved its goal as a long-term treatment for patients with allograft dysfunction. The long-term outcome of graft and patient survival is highly influenced by the occurrence of chronic rejection. The dominant pathological features of chronic rejection are persistent perivascular inflammatory cell infiltration, generalized transplant arteriosclerosis characterized by concentric neointimal formation and vascular occlusion, and interstitial fibrosis. In addition, long-term use of calcineurin inhibitors results in nephrotoxicity, hypertension, and hyperlipidemia, and these persistent adverse effects could further accelerate the formation of transplant arteriosclerosis. Although considerable effort has been invested to prevent transplant arteriosclerosis in experimental and clinical studies, an effective strategy for preventing transplant arteriosclerosis has not been established.

Recently, FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride) has been discovered by structural modification of myriocin (ISP-1) isolated from the culture broth of the fungus Isaria sinclairii. FTY720 has unique properties unlike those
of calcineurin inhibitors or corticosteroids, and administration of FTY720 sequesters peripheral lymphocytes into the secondary lymphoid organ, resulting in immunosuppressive effects in organ transplantation. In the resultant study, the first human clinical trials of FTY720 were performed, and the efficacy of this new type of immunosuppressant in renal transplant patients was reported, but the occurrence of asymptomatic bradycardia was observed as the most common adverse event. We have recently discovered and synthesized a novel immunosuppressant, KRP-203 (2-amino-2-[2-[4-(3-benzoyloxyphenylthio)-2-chlorophenyl]ethyl]-1,3-propanediol hydrochloride, molecular weight 480.45 Da), that has some similarity to the molecular structure of FTY720 (Figure 1). The structural similarity of KRP-203 to that of FT720 suggests that this agent may also sequester peripheral lymphocytes and accelerate lymphocyte homing into the secondary lymphoid organ. In organ transplantation, alloimmune response by infiltration of mononuclear cells, including lymphocytes, accelerates allograft rejection. Therefore, we tested whether KRP-203 could have an immunosuppressive potential by accelerating the lymphocyte homing and reducing intragraft immune response by lymphocyte infiltration in rat transplant models. Furthermore, we addressed whether KRP-203 could attenuate chronic rejection in a rat heart allograft model and diminish the occurrence of bradycardia in the Hartley guinea pig model.

**Methods**

**Experimental Animals and Immunosuppressive Agents**

Inbred male DA (MHC haplotype: RT1b) rats were obtained from Japan SLC Inc (Hamamatsu, Japan). The inbred male LEW (RT1l) guinea pig model.

**Rat Skin and Heart Allograft Models**

Rat skin transplantation was performed using 5-week-old male rats according to Taylor’s procedure. The skin of LEW rat was transplanted to the lateral thorax of F344 rat. The allografts were observed daily, and rejection was defined as complete arrest. Rejection was defined as a heartbeat of grade 0 or 1. KRP-203 and FTY720 were administered orally. Control animals were given only the vehicle. Skin and heart allograft survival was followed up until the day of rejection or for 30 days and until the day of rejection or for 100 days after transplantation, respectively.

Treatment with KRP-203 alone or in combination with CsA was tolerated well. Rats in all groups showed a similar weight gain, and no abnormal behavior was observed. No clinically detectable infections occurred during the observation period.

**Laboratory Data Analysis**

Blood samples obtained from the recipients with heart allografts for hematological and biochemical analyses were measured by SRL, Inc.

**Histological Examination**

Transverse paraffin sections 4 μm thick were stained with hematoxylin and eosin and Masson’s trichrome. The severity of acute rejection was scored using a previously described scoring system. To quantify the degree of neointimal formation in the coronary arteries of heart allografts, the percentage of vascular occlusion was calculated using NIH image 1.64, as follows: vascular occlusion (%) = (area of intima/ (area of intima + vascular lumen) × 100. The numbers of coronary arteries and grafts examined were as follows (arteries and grafts, respectively): control group, 50 and 6; KRP-203 0.1 mg · kg⁻¹ · d⁻¹ group, 30 and 4; and KRP-203 1 mg · kg⁻¹ · d⁻¹ group, 51 and 6. In addition, the degree of perivascular inflammatory cell infiltration was evaluated as follows: grade 0, no infiltration; grade 1, focal infiltration; grade 2, moderate infiltration; and grade 3, diffuse infiltration. Sections stained with Masson’s trichrome were analyzed for the degree of myocardial fibrosis. The scoring for the degree of myocardial fibrosis in each section was defined as follows: grade 0, no fibrosis; grade 1, focal fibrosis; grade 2, moderate fibrosis; grade 3, diffuse fibrosis. Four areas (right wall, anterior wall, posterior wall, and septum) were analyzed from each section, and the results were averaged.

**Immunohistochemical Analysis**

Immunohistochemical staining was performed using the peroxidase-antiperoxidase or streptavidin-biotin method. The sections were stained with Masson’s trichrome were analyzed for the degree of myocardial fibrosis. The scoring for the degree of myocardial fibrosis in each section was defined as follows: grade 0, no fibrosis; grade 1, focal fibrosis; grade 2, moderate fibrosis; grade 3, diffuse fibrosis. Four areas (right wall, anterior wall, posterior wall, and septum) were analyzed from each section, and the results were averaged.

**Flow Cytometry Analysis**

Peripheral blood was obtained from male Wistar rats (7 weeks old) at 24 hours after the administration of saline (control), 0.1 mg · kg⁻¹ · d⁻¹ KRP-203, or 1 mg · kg⁻¹ · d⁻¹ KRP-203 (each group, n = 3). The peripheral blood cells were stained with FITC-conjugated anti-rat CD3 (clone: 1F4, Oxford Biotechnology Ltd) and phycoerythrin-conjugated anti-rat CD45RA (clone: OX-33, Cosmo Bio) monoclonal antibodies. The numbers of white blood cells (WBCs), lymphocytes, monocytes, granulocytes, CD3-positive cells, and CD45RA-positive B cells were determined by FACScan (BD Biosciences).

To investigate the homing of peripheral lymphocytes into peripheral lymph nodes, we used CFSE dye (Dojin) as a marker. Peripheral blood mononuclear cells obtained from LEW rats (12 weeks old) were suspended in PBS and labeled with 2.5 μmol/L CFSE dye for 5 minutes at room temperature. After washing, CFSE-labeled peripheral blood mononuclear cells (13.4 × 10⁶ cells) were injected into the tail vein of the recipient LEW rats (10 weeks old, n = 6). The recipient rats were given 1 mg · kg⁻¹ · d⁻¹ KRP-203 (n = 3) or vehicle (n = 3) orally 24 hours before the cell injection. At 1 hour after the cell injection, axillar, mesenteric, and submandibular lymph nodes were obtained and analyzed by FACScan.
Determination of Heart Rate in Anesthetized Guinea Pigs

The effects of KRP-203 and FTY720 on heart rate were examined in anesthetized Hartley guinea pigs (each group; n=6). KRP-203 (0.03 mg·kg⁻¹·d⁻¹) or FTY720 (0.03 mg·kg⁻¹·d⁻¹) was injected into the external jugular vein while the ECG was monitored.

Statistical Analysis

Statistical analysis was performed by use of Stat View version 5.0 (Abacus Concepts Inc). In rat skin and heart allograft models, survival time was compared between groups by the log-rank test.

KRP-203 Has a High Immunosuppressive Potential in mHC-Disparate Rat Skin Allografts

We first examined the effect of KRP-203 and FTY720 on mHC-disparate rat skin allograft survival. In this combination of LEW to F344, KRP-203 prolonged graft survival and attenuated chronic rejection in mHC-disparate rat heart allografts.

Statistical Analysis

Statistical analysis was performed by use of Stat View version 5.0 (Abacus Concepts Inc). In rat skin and heart allograft models, survival time was compared between groups by the log-rank test. All differences were defined as significant at a value of P<0.05.

Results

KRP-203 Has a High Immunosuppressive Potential in mHC-Disparate Rat Skin Allografts

We first examined the effect of KRP-203 and FTY720 on mHC-disparate rat skin allograft survival. In this combination, all allografts in the control group were rejected at approximately 8 to 9 days after transplantation (Table 1), whereas treatment with KRP-203 significantly prolonged the skin graft survival (P<0.01). The mean survival time (MST) of the KRP-203–treated group at a dose of 0.3, 1.0, and 3.0 mg·kg⁻¹·d⁻¹ was >17.4, >27.0, and >27.4 days, respectively. FTY720 treatment also enhanced the skin allograft survival, but the MST of the group treated with FTY720 at a dose of 0.3, 1.0, and 3.0 mg·kg⁻¹·d⁻¹ was >19.0, >21.2, and >24.6 days, respectively.

KRP-203 Prolonged Graft Survival and Attenuated Chronic Rejection in mHC-Disparate Rat Heart Allografts

We next examined the effect of KRP-203 on mHC-disparate rat heart allograft survival. In the control rats, 3 of 8 allografts had complete heartbeat cessation (grade 0) at 21, 24, and 53 days after transplantation, respectively (Figure 2A). Four of 5 allografts were grade 1 at 74, 79, 87, and 94 days, respectively. In contrast, all allografts survived without rejection after treatment with KRP-203 at 1 and 0.1 mg·kg⁻¹·d⁻¹. The heartbeat score was significantly higher in the KRP-203–treated rats than in the control rats (Figure 2B). Furthermore, the marked enlargement of grafted hearts observed in the control rats was absent in KRP-203–treated rats (Figure 3A). The histological and immunohistochemical examinations revealed that marked vascular occlusion; perivascular infiltration of mononuclear cells, including macrophages and T cells; and myocardial fibrosis were observed in the control rats, whereas all these findings were significantly attenuated in the rats treated with both 0.1 and 1 mg·kg⁻¹·d⁻¹ KRP-203 (Figures 3B and 4). We further analyzed ET-1 expression in coronary arteries of allografts, because ET-1 is a key molecule for the formation of transplant arteriosclerosis. In the control rats, remarkable expression of ET-1 in the neointimal lesion of coronary arteries, TGF-β1 in the infil-
trated cells and myocardium, and marked IgG deposition in neointimal and perivascular lesions were observed, whereas the expression of these molecules was inhibited in KRP-203–treated rats (Figure 3, B and C).

KRP-203 Prolongs Heart Allograft Survival in MHC-Incompatible Rat Combination

The effect of KRP-203 on MHC-incompatible rat heart allograft survival was also examined. The allografts in the control group were rejected at approximately 6 to 7 days after transplantation (Table 2), whereas treatment with either KRP-203 or FTY720 prolonged the allograft survival. The MSTs of the KRP-203 and FTY720 groups were 9.7 and 7.8 days, respectively. The histological grade scores for acute rejection were not significantly different among the control, KRP-203, and FTY720 groups. In laboratory data, WBC counts decreased in both the KRP-203 and the FTY720 treatment groups compared with the control group (Table 3). However, no progressive anemia was observed in the treatment groups. There were no significant differences in platelet counts or the serum levels of alanine aminotransferase, blood urea nitrogen, creatinine, triglyceride, and total cholesterol between these groups.

Because the efficacy of KRP-203 was not very significant in MHC-incompatible rat heart allograft survival, we further examined the synergistic effect of KRP-203 combined with a low dose of CsA. Treatment with CsA 3 mg·kg⁻¹·d⁻¹ alone prolonged the allograft survival, but the MST of the CsA group
was 12.5 days. In contrast, KRP-203 1 mg \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1} \) treatment in combination with CsA 3 mg \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1} \) markedly prolonged the allograft survival, and the MST was >30 days. Histology also showed that this combination treatment strikingly prevented severe acute rejection characterized by diffuse infiltration of macrophages and T cells, severe hemorrhage, and myocardial necrosis (Data Supplement Figure, A and B). The histological grade scores for rejection in this treatment were also significantly less than in any other treatments (\( P<0.05 \)).

**KRP-203 Decreases Peripheral Circulating Lymphocytes and Accelerates Lymphocyte Homing Into Peripheral Lymph Nodes**

To address the potential mechanisms of the immunosuppressive effects of KRP-203, we first examined a peripheral WBC subpopulation from rats at 100 days after heart transplantation in an mHC-disparate combination. In KRP-203–treated rats, total peripheral WBC counts decreased by 50% to 60% compared with vehicle-treated rats (Figure 5A). The WBC subpopulation analysis revealed that the percentage of lymphocytes was remarkably decreased by the treatment with KRP-203, whereas the percentage of granulocytes was relatively increased. There was no significant difference in the percentage of monocytes and eosinocytes between the KRP-203–treated group and the control group.

The effect of a single administration of KRP-203 on the number of peripheral lymphocytes was also evaluated by flow cytometric analysis. The oral administration of 0.1 or 1 mg \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1} \) KRP-203 caused a significant reduction of the substantial number of lymphocytes. CD3-positive T cells, CD45RA-positive B cells, and monocytes decreased in a dose-dependent manner, but granulocytes were not affected (Figure 5B). In particular, CD3-positive T cells were completely diminished.

Because peripheral lymphocytes were found to be diminished by treatment with KRP-203, we further tested whether KRP-203 induced homing of peripheral blood lymphocytes into peripheral lymph nodes by CFSE dye–stained lymphocytes. As shown in Figure 5C, a single administration of 1 mg \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1} \) KRP-203 obviously induced lymphocyte homing into axillar, mesenteric, and submandibular lymph nodes.

**KRP-203 Reduces the Occurrence of Bradycardia in Hartley Guinea Pigs**

Because FTY720 has been shown to induce bradycardia as an adverse effect in human clinical studies,\(^7\) we evaluated whether KRP-203 induced bradycardia in an anesthetized Hartley guinea pig model. When 0.03 mg \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1} \) FTY720 was administered intravenously, heart rate rapidly declined within 15 to 30 minutes (Figure 6). This bradycardia was

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Quantitative analysis of vascular occlusion (A), perivascular inflammatory cell infiltration (B), and myocardial fibrosis (C) in mHC-disparate heart allografts. Data shown are mean±SEM. Quantitative analysis of intimal and adventitial infiltration of macrophages (D) and T cells (E). Data shown are mean±SEM. The numbers in parentheses indicate the dose (mg \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1} \)) of immunosuppressive agents. *\( P<0.05 \) vs control group.

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### TABLE 2. Synergistic Effect of KRP-203 With CsA on Heterotopic Heart Allograft Survival in a Rat Combination of DA to LEW

<table>
<thead>
<tr>
<th>Treatment (dose, mg·kg(^{-1} \cdot d^{-1} ))</th>
<th>n</th>
<th>Graft Survival, d</th>
<th>MST, d</th>
<th>Histological Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syngeneic</td>
<td>6</td>
<td>&gt;30, &gt;30, &gt;30, &gt;30, &gt;30, &gt;30</td>
<td>&gt;30†</td>
<td>0.00±0.00§</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>6</td>
<td>6, 6, 6, 6, 6, 7</td>
<td>6.2</td>
<td>3.00±0.00</td>
</tr>
<tr>
<td>KRP-203 (1)</td>
<td>6</td>
<td>8, 8, 9, 11, 11, 11</td>
<td>9.7†</td>
<td>2.67±0.21</td>
</tr>
<tr>
<td>FTY720 (1)</td>
<td>6</td>
<td>7, 7, 8, 8, 8, 9</td>
<td>7.8*</td>
<td>2.83±0.17</td>
</tr>
<tr>
<td>CsA (3)</td>
<td>6</td>
<td>7, 8, 11, 12, 17, 20</td>
<td>12.5†</td>
<td>2.50±0.50</td>
</tr>
<tr>
<td>KRP-203 (1) + CsA (3)</td>
<td>6</td>
<td>25, &gt;30, &gt;30, &gt;30, &gt;30, &gt;30</td>
<td>&gt;30‡</td>
<td>1.00±0.57§</td>
</tr>
</tbody>
</table>

MST indicates mean survival time.

*\( P<0.005 \) vs control; †\( P<0.005 \) vs control or FTY720; ‡\( P<0.001 \) vs control, FTY720 (1), KRP-203 (1), or CsA (3); §\( P<0.05 \) vs control, FTY720 (1), KRP-203 (1), or CsA.
sustained for 75 minutes and then gradually recovered at 90 minutes. In contrast, no induction of bradycardia was observed in the same dose treatment of KRP-203.

**Discussion**

The major findings of this study are the following: (1) KRP-203 significantly prolonged mHC-disparate rat skin allografts; (2) KRP-203 markedly prolonged mHC-disparate rat heart allografts, especially attenuated chronic rejection, along with preventing neoimtional formation, infiltration of inflammatory cells, including macrophages and T cells, endothelin-1 and TGF-β expression, IgG deposition, and myocardial fibrosis; (3) KRP-203 slightly prolonged MHC-incompatible rat heart allografts, but KRP-203 combined with a low dose of CsA synergistically prolonged the allograft survival; (4) KRP-203 reduced the number of peripheral blood leukocytes (lymphocytes and monocytes but not granulocytes) and accelerated lymphocyte homing into peripheral lymph nodes; and (5) KRP-203 showed less of a tendency to cause bradycardia than FTY720. These findings suggest that KRP-203 could be a more suitable therapeutic agent in patients with organ transplantation.

Acute rejection in allogeneic solid organ transplantation is a cell-mediated pathological inflammatory response, which usually occurs within the first few months after transplantation. In recent years, it has been possible to treat and prevent acute rejection adequately with immunosuppressive agents such as CsA and FK506. We demonstrated that KRP-203 prolonged MHC-incompatible rat heart allograft survival compared with FTY720, but the prolonged effect was not so potent. Thus, our results suggest that KRP-203 or FTY720 monotherapy would be effective but insufficient to inhibit the acute alloglume response. We therefore tested the synergistic effect of KRP-203 combined with CsA in heart allografts. Because a high or even therapeutic dose of CsA has substantial adverse vascular effects that may give rise to nephrotoxicity, hypertension, and hyperlipidemia, only subtherapeutic doses of CsA were used in this study. Combination treatment with KRP-203 with a subtherapeutic dose of CsA clearly prolonged the allograft survival and attenuated the allograft vasculopathy. Thus, this combination would be expected to be a promising strategy for preventing allograft rejection and reducing adverse effects.

Chronic rejection is now recognized as the leading cause of late graft loss and patient death after the first year after organ transplantation. The incidence of chronic rejection after transplantation depends on the type of organ grafted and varies from 30% to 50% in kidney allografts, to >50% in heart allografts, and to 70% in lung allografts 5 years after transplantation.1,3,4 The histopathological characteristics associated with chronic rejection vary between the different organs, but there is a common histomorphological feature of transplant arteriosclerosis characterized by neointimal formation, perivascular inflammatory cell infiltration, and interstitial fibrosis.1,3 At present, however, successful strategies to prevent chronic rejection have not been established. Our study demonstrated that KRP-203 successfully prevented infiltration of perivascular inflammatory cells, including macrophages and T cells, vascular occlusion, and myocardial fibrosis 100 days after heart transplantation in a rat model. Immunohistochemical analysis further revealed that KRP-203 inhibited the expression of ET-1 in coronary arteries and TGF-β1 in infiltrated inflammatory cells of the allografts. Both ET-1 and TGF-β1 are produced by macrophages and are known as key mediators for transplant arteriosclerosis.12,13 Because KRP-203 diminished infiltration of inflammatory cells, the decrease of these cells by KRP-203 might inhibit ET-1 and TGF-β1 production, resulting in abrogation of transplant arteriosclerosis.

Because KRP-203 has a similarity to FTY720, we examined whether KRP-203 induces lymphocyte homing into peripheral lymph nodes and reduces peripheral WBC counts. As expected, KRP-203 induced lymphocyte homing into peripheral lymph nodes and reduced the number of total WBCs in peripheral blood. In particular, KRP-203 reduced the number of CD3-positive T cells, CD45RA-positive B cells, and monocytes. Interestingly, a low dose of KRP-203 (0.1 mg·kg⁻¹·d⁻¹) reduced not only CD3-positive T cells but also CD45RA-positive B cells in peripheral blood, whereas FTY720 at a dose of 0.3 mg·kg⁻¹·d⁻¹ has no effect on CD45RA-positive B cells.14 Consistent with these findings, we observed that KRP-203 reduced IgG deposition in the neointimal and perivascular lesions of the heart allograft. There are emerging reports that humoral immune responses that are mediated by B cells may play an important role in the development of transplant arteriosclerosis.15,16 Taken together, the findings obtained from this study suggest that KRP-203 prevents both cellular and humoral immune responses that are essential for the development of transplant arteriosclerosis.

The first clinical trials of FTY720 have recently reported the efficacy and safety of this agent in patients with renal

### TABLE 3. Effect of KRP-203 Combined With CsA on Laboratory Data

<table>
<thead>
<tr>
<th>Treatment (dose, mg·kg⁻¹·d⁻¹)</th>
<th>n</th>
<th>WBC, ×10⁹ μL</th>
<th>Hb, g/dL</th>
<th>Hct, %</th>
<th>Plt, ×10⁹ μL</th>
<th>ALT, IU/L</th>
<th>BUN, mg/dL</th>
<th>Cr, mg/dL</th>
<th>TG, mg/dL</th>
<th>T-Chol, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syngenic</td>
<td>6</td>
<td>12.3±1.6</td>
<td>14.7±0.2*</td>
<td>48.8±1.3*</td>
<td>76.5±5.7</td>
<td>ND</td>
<td>19.8±0.8</td>
<td>0.27±0.02</td>
<td>120±7.5</td>
<td>76.6±6.8</td>
</tr>
<tr>
<td>Control (vehicle)</td>
<td>6</td>
<td>14.1±1.6</td>
<td>12.1±0.9</td>
<td>38.5±2.4</td>
<td>70.7±8.4</td>
<td>48.7±12.5</td>
<td>19.4±1.8</td>
<td>0.33±0.04</td>
<td>109±12.1</td>
<td>75.2±6.6</td>
</tr>
<tr>
<td>KRP-203 (1)</td>
<td>6</td>
<td>7.3±0.9†</td>
<td>13.9±0.3*</td>
<td>45.1±1.7*</td>
<td>69.2±10.4</td>
<td>47.7±20.6</td>
<td>18.5±1.7</td>
<td>0.29±0.04</td>
<td>106±37.0</td>
<td>74.2±6.5</td>
</tr>
<tr>
<td>FTY720 (1)</td>
<td>6</td>
<td>7.3±1.2†</td>
<td>14.0±0.5*</td>
<td>43.7±2.3*</td>
<td>61.3±8.0</td>
<td>48.7±5.2</td>
<td>19.2±2.2</td>
<td>0.30±0.04</td>
<td>104±37.0</td>
<td>78.0±5.7</td>
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<tr>
<td>CsA (3)</td>
<td>6</td>
<td>18.0±1.6</td>
<td>14.2±0.4*</td>
<td>45.6±1.0*</td>
<td>88.6±10.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>KRP-203 (1) + CsA (3)</td>
<td>6</td>
<td>4.2±0.9†</td>
<td>15.3±0.2*</td>
<td>48.0±1.0*</td>
<td>73.3±4.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

WBC indicates white blood cell; HB, hemoglobin; Hct, hematocrit; Plt, platelet; ALT, alanine aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; TG, triglyceride; T-Chol, total cholesterol; and ND, not determined.

*P<0.05 vs control; †P<0.05 vs syngeneic, control, or CsA (3).
Figure 5. A, Total WBC numbers and subpopulations in peripheral blood in mHC-disparate heart allografts at 100 days after transplantation. B, Effect of KRP-203 on number of WBCs in peripheral blood 24 hours after administration. C, Effect of KRP-203 on lymphocyte homing into peripheral lymph nodes 24 hours after administration. Data shown are mean±SEM. The numbers in parentheses indicate the dose (mg·kg⁻¹·d⁻¹) of immunosuppressive agents. LN indicates lymph node. *P<0.05, **P<0.01, and ***P<0.001 vs control group.
transplantation? The occurrence of bradycardia, however, was reported as the most common adverse event. The FTY720-associated bradycardia was transient and asymptomatic, but it occurred in 42% of patients. In this study, we found that KRP-203 clearly showed less of a tendency to cause bradycardia than FTY720, suggesting that KRP-203 has fewer adverse effects than FTY720. Recent studies found that KRP-203 clearly showed less of a tendency to cause bradycardia than FTY720, suggesting that KRP-203 offers considerable potential as a novel therapeutic immunosuppressant not only to prevent graft rejection but also to reduce adverse effects in patients with organ transplants.

Acknowledgments
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References

![Figure 6. Influence of FTY720 and KRP-203 on heart rate in anesthetized guinea pigs. Data shown are mean ± SEM. The numbers in parentheses indicate the dose (mg · kg⁻¹ · d⁻¹) of immunosuppressive agents. *P < 0.05 vs control group.](http://circ.ahajournals.org/content/122/11/2291/F6.large.jpg)
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